ACTA MEDICA SCANDINAVICA

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VOLUME 175 1964

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Haemodynamics in Acute Myocardial Infarction

By

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High mortality in acute myocardial infarction has stimulated several investigators (1 2 3 4 10 and 12) to perform haemodynamic studies in patients despite the risks involved. These studies have generally shown decreased cardiac output and normal peripheral resistance during the acute stage of moderately severe infarction. Low cardiac output and normal or high resistance was found in patients with shock. The mean cardiac output was again higher at the end of hospital stay

The course of haemodynamic events in a given patient during the first few most vulnerable days is, however difficult to assess from those data because the results vary considerably between laboratories and because requisite information on body temperature t the time of the study is lacking. Day-to-day study may help us to understand the basis for the development of heart failure or cardiac shock. The haemodynamic influence of body temperature is considerable in normal men (5) and may be even more important in patients with myocardial infarction. These considerations prompted the present study. Haemodynamic data for the first 3 days of infarction are presented in this paper and compared with data from the same patients at the end of hospital stay as well as with similar data for controls.

Material

The material comprised 3 females and 38 males aged 37 to 77 mean 56, years. All were inhabitants of Gothenburg where nearly all patients with suspected myocardial infarction are admitted to the same hospital within hours. Those investigated in the acute stage were selected from patients in this hospital because they showed clear evidence of myocardial infarction during the first or the first few days of admittance.

Hypertension was previously known in 5 patients, diabetes mellitus in 2 and cholecystopathy in 2. One patient had myocardial infarction 3 months earlier and was admitted to hospital for recurrence. Four patients had had effort angina prior to infarction.

ECGs wer routinely taken on the 1st, 2nd, 3rd and about 7th, 14th and 21st day. All patients included here had electrocardiographic signs of infarction. There were 25 patients with predominantly anterior infarctions and 16 with predominantly posterior infarctions.

All but 5 patients had temperature rise in the first days. I all but one patient a rise in serum GOT was found.

The systolic blood pressure fell to 90 mm Hg or less in 13 patients. Ten other patients had

Submitted for publication June 11 1963.

Table I Mean values of haemodynamic data of male patients with sinus rhythm on the 1st, 2nd and 3rd day of myocardial infarction

Myocardial Infarction	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
				Systolic	Diastolic	Mean			
Controls									
No.	12	12	12	12	12	12	12	12	12
Mean ± SE of mean.	68±2	5.7±0.2	83±3	139±7	71±3	98±4	18±1	7.7±0.6	114±7
SD	9	0.8	11	23	11	15	3	1.9	26
1st day									
No.	10	10	10	10	10	10	10	10	10
Mean ± SE of mean	79±7	5.3±0.2	72±5	125±6	68±5	92±6	18±1	6.7±0.5	88±7
SD	21	0.6	17	20	15	18	3	1.6	22
2nd day									
No.	21	21	21	21	21	21	21	21	21
Mean ± SE of mean	79±4	5.4±0.2	71±4	116±3	62±2	84±2	16±1	6.1±0.3	79±4
SD	18	1.0	19	13	10	10	3	1.3	17
3rd day									
No.	18	18	18	18	18	18	18	18	18
Mean ± SE of mean	87±4	5.3±0.3	63±4	110±3	58±2	79±2	16±1	5.7±0.3	68±5
SD	18	1.1	16	12	9	10	4	1.3	20
Probabil. of diff. between controls and pat. on 1 day of infarction	–	–	< 0.05	–	–	–	–	–	< 0.05
Probabil. of diff. between 1st and 3rd day of infarction	–	–	–	< 0.05	–	< 0.05	–	–	< 0.02

separately. One patient with a cardiac output of 10.8 l the first day of infarction and another with a cardiac output of 11.. l prior to discharge are excluded. It has not been possible to find the cause of those high outputs but they would have distorted the means.

Cardiac output for controls was 5 7 l/min., stroke volume 83 ml, brachial arterial pressures 139/71 mean 98, mm Hg, resistance 18 units, left ventricular work 7 7 kpm/min. and left ventricular stroke work 114 pm.

ACUTE STAGE

Haemodynamic changes over the first 3 days of infarction

Patients investigated on the first day of myocardial infarction had usually no temperature measurement in the mor-

decreases in systolic pressure of 50 mm Hg or more, measured auscultatorily during the first days in hospital.

Recurrent pain in the chest occurred in 12 patients during the first few days. Sixteen patients had symptoms or signs of heart failure during their hospital stay

Three patients sometimes had pains in the chest during the investigation and five had pains and cold sweat and were given analgetics. None had dyspnoea or signs of pulmonary oedema during the investigation.

Seven patients died in the hospital. Autopsy confirmed the clinical diagnosis in all cases.

In 33 patients an X ray was taken of the chest before they left hospital. Ten had a heart volume greater than 450 ml/sqm body surface area, measured according to Jonsell (6) Seven had signs of pulmonary stasis.

Of those who were discharged six are known to have died. The majority have returned to gainful occupations.

Twelve male subjects aged 50 to 67 mean 57 years served as controls. Ten were in hospital for diseases not affecting the cardiovascular system and two were volunteers. One complained of exertional dyspnoea and another was admitted to hospital for paroxysmal atrial fibrillation one year after he had been accepted as a control. They were investigated recumbent by the same technique as for the patients.

Methods

Catheters were inserted percutaneously into the brachial artery and into the cubital vein (11) The tip of the vein catheter was advanced to the subclavian vein. Arterial pressures were recorded with a transducer of the variable inductance type (Elema Co) Mean pressures were obtained by electric integration. Pressures refer to a point 5 cm below the sternal notch. Pressures and electrocardiograms were recorded on an ink-jet writing apparatus (Elema Mingograf 42 B) The cardiac output was determined by dye dilution technique, with bromsulphalein as indicator (14) Hematocrit was measured after centrifuging at 5 000 r p. m. for 15 minutes.

Expired air was collected in a Douglas bag and analysed for oxygen. Arteriovenous oxygen difference was calculated from the cardiac output and the oxygen consumption.

Peripheral resistance was calculated as the mean pressure in the brachial artery divided by the cardiac output and is given in arbitrary units.

Left ventricular work was calculated as the brachial arterial mean pressure times the cardiac output and is expressed as kpm/min. Left ventricular stroke work was calculated as the mean pressure in the brachial artery times the stroke volume and is expressed in pond-metres (pm)

Means, standard deviations and standard errors of the means were calculated according to standard methods. Multivariate regressions were analyzed (13) Differences were tested with Student's t-test. All test levels are 5 % or lower

Procedure

Patients arriving in the hospital in the morning with unequivocal signs of myocardial infarction were investigated in the afternoon. Subsequent investigations were made in the morning Some of the patients had a light breakfast before the investigation. All investigations in the acute stage were made in the ward, and those before the patient left the hospital were made in the cardiopulmonary laboratory In the acute stage the patients were investigated in the recumbent position with their head and sometimes chest comfortably elevated. The other investigations were made with the patient recumbent and then sitting or either position.

The first cardiac output determination was made about 30 minutes after insertion of the catheters, the second 20—40 minutes later In a minority of the patients the blood loss of about 40 ml at each determination was replaced by stored blood.

In 22 patients the catheters were left in place for 24 to 48 hours. The catheters were rinsed every two hours with a solution of heparin in isotonic saline, 50 mg/100 ml. The patients were under constant supervision.

Results

Means refer only to men except for means of differences which include values for two females Values for one female and one male with arrhythmias are given

1st to the 3rd day and the mean pressure by 7.5 mm Hg ($p < 0.02$). Resistance fell by 1.9 units ($p < 0.01$). Left ventricular work or stroke work did not decrease significantly. These data are also given in the first part of table V.

Haemodynamic changes and temperature

There were 22 duplicate or quadruplicate observations on circulation when temperature was below 38° C and 16 when the temperature was higher during the first three days of infarction. The mean temperatures in those two groups were 37.5° C and 38.4 C. The mean heart rate and mean cardiac output were higher ($p < 0.02$ and < 0.05) when the temperature was higher the brachial arterial systolic and mean pressures and resistance were lower ($p < 0.02$, < 0.05 and < 0.001). The stroke volume, left ventricular work and left ventricular stroke work were unchanged. The values are given in table II.

Cardiac output and temperature is plotted in fig. 1. The relation between cardiac output and temperature is poor in the individual patients.

Resistance and temperature are given in fig. 2. The relation between rise in temperature and fall in resistance is obvious, even in individual cases.

The influence of temperature can be analyzed partly separated from the influence of time by comparing patients with a temperature less than 38° C on the third day of infarction and patients with a higher temperature on the same day.

The morning temperature on the 3rd day was less than 38° C in 7 male patients and higher in 11. The former had a heart rate of 80, a cardiac output of 4.7 l/min. arterial blood pressures 111/59, mean 81 mm Hg and resistance of 18 units. The corresponding values for those with

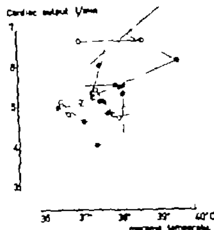


Fig. 1. Cardiac output in relation to morning temperature in male patients during first three days of myocardial infarction. Values for the first day are symbolized by unfilled circles, second-day values by semi-filled circles and third-day values by filled circles. Symbols belonging to the same patient are connected by lines.

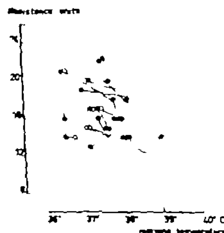


Fig. 2. Peripheral vascular resistance in relation to morning temperature in male patients during the first three days of myocardial infarction. Same symbols as in fig. 1.

higher temperature were 91, 5.6 l/min. 109/58, mean 78, mm Hg and 14 units. The cardiac outputs and resistances differed significantly (both $p < 0.05$)

Table II Mean values of haemodynamic data of male patients with sinus rhythm on the 1st, 2nd and 3rd day of myocardial infarction in relation to the morning temperature

Morning temp.	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)	Brachial arterial pressure (mm Hg) Systolic	Diastolic	Mean	Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
< 38 C									
No.	22	22	22	22	22	22	22	22	22
Mean ± SE of mean	79±4	5 0±0.2	67±3	120±4	65±3	87±3	18±1	6.1±0.3	79±4
SD	17	0.8	13	17	13	13	3	1.3	19
≥ 38 C									
No.	16	16	16	16	16	16	16	16	16
Mean ± SE of mean	94±4	5 7±0.2	64±4	108±3	60±2	80±2	14±1	6.2±0.3	69±5
SD	17	0.9	15	10	6	7	2	1.2	18
Probabl. of diff	< 0.02	< 0.05	—	< 0.02	—	< 0.05	< 0.001	—	—

ning but the temperature on admittance ranged from 36 0 to 37 5 C, mean 37 0 Patients investigated on the 2nd day had morning temperatures between 36.5 and 39 6° C mean 37 7 C. Patients investigat ed on 3rd day had morning temperatures ranging from 36 0 to 39 0 C, mean 38 0 C All temperatures were recorded rectally

Mean heart rates on the first three days were 79 79 and 87 There was no significant difference between these val ues. Mean cardiac outputs were 5 3 5 4 and 5.3 l/min and mean stroke volumes 72 71 and 63 ml There were no signif icant differences between these values. Stroke volume was lower already on the 1st day of infarction when compared with controls ($p < 0\ 05$)

Mean brachial arterial systolic pressure decreased from 125 to 116 to 110 mm Hg ($p < 0\ 05$) over the first three days Diastolic pressures were 68 62 and 58 mm Hg and the means of the mean pressures decreased from 92 to 84 to 79 mm Hg ($p < 0\ 05$)

Mean total peripheral resistances were 18 16 and 16 units and mean left ventricular works 6 7 6 1 and 5 7 kpm/ min. on these 3 days.

Left ventricular stroke work decreased from 88 to 79 to 68 pm ($p < 0\ 02$) over these days and already on the 1st day of infarction it was lower ($p < 0\ 05$) than in the controls.

Circulatory data for controls and male patients on the 1st to 3rd days of in farction are given in table I There were significant differences between controls and patients on the 1st day of infarction and between patients on the 1st and 3rd days of infarction

Nine patients were investigated on the 1st and 2nd days of infarction and 12 on the 2nd and 3rd days. Changes in the circulation from the 1st to the 2nd day and from the 2nd to the 3rd day were similar Data for these patients were treated together

The systolic pressure decreased by 8 3 mm Hg ($p < 0\ 05$) per day from the

on these days and of patients with temperature 38° C or higher on 3rd day (means and SE of means)

Brachial arterial pressure (mm Hg)						Resistance (units)		Left ventricular work kpm/min)		Left ventricular strokework(pm)	
Systolic		Diastolic		Mean							
1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd
127 ± 7	111 ± 5	68 ± 5	59 ± 4	92 ± 6	81 ± 4	18 ± 1	18 ± 1	6.8 ± 0.6	5.2 ± 0.5	92 ± 7	68 ± 8
	109 ± 3		58 ± 2		78 ± 3		14 ± 1		6.0 ± 0.3		69 ± 6
	—		—		—		< 0.05		—		—
	< 0.01		< 0.05		< 0.02		—		< 0.01		< 0.001

= day of infarction (1st, 2nd or 3rd) t = temperature (C in the morning)

Cardiac output fell about 0.3 l/min. per day over the first three days, and rose about 0.6 l/min. per degree rise in temperature

Regression analysis of resistance and day of infarction did not show any significant correlation. Resistance and morning temperature were significantly correlated ($p < 0.02$) ($r = -0.35$) The estimated function is

$$R = 75.4 - 1.57\,t$$

where R = resistance (author units)
t = temperature (C in the morning)

Resistance is not influenced by time elapsed after myocardial infarction but falls about 1 6 units per degree rise in temperature.

Interrelation of circulatory functions

The day-to-day changes in cardiac output for individual patients seem not to be closely related to changes in brachial arterial mean pressure (fig 3)

There were, however 6 observations during the first 5 days on patients with mean arterial blood pressure below 70 mm Hg Five had a rather low cardiac output with a mean of 4 1 l/min. while the sixth had an output of 7 0 l/min.

The changes in resistance from day to day of individual patients showed a close relation to changes in brachial arterial mean pressure (fig 4)

There were 14 observations in 11 patients of a drop in mean arterial blood pressure of 7 per cent or more between investigations on the 1st and 2nd, 2nd and 3rd or 3rd and 4th day of myocardial infarction. The fall in brachial arterial mean pressure was accompanied by a rise in cardiac output in 4 patients. In two of them the temperature simultaneously rose from 37 1 to 39 6 and from 37.2 to 39 0 C. Resistance increased in one patient and fell in all the other patients.

These changes are depicted in fig 5

Brachial arterial systolic pressure and pulse pressure can readily be measured with adequate accuracy in most patients. The relation of those parameters to blood flow and resistance is thus of special interest.

When the systolic pressure during the first 3 days of myocardial infarction was above 120 mm Hg the resistance was

Table III Comparison of circulatory data for 1st and 3rd day of patients with temperature less than 38 C
(The number of patients in the groups were 9, 7 and 11)

Temp.	Heart rate (beats/min)		Cardiac output (l/min)		Stroke vol. (ml)	
	1st	3rd	1st	3rd	1st	3rd
< 38 C	76 ± 7	80 ± 6	5.4 ± 0.2	4.7 ± 0.4	74 ± 5	60 ± 6
≥ 38 C		91 ± 6		5.6 ± 0.3		65 ± 5
Probabil. of diff between high and low temp. on 3rd day		–		< 0.05		–
Probabil. of diff. between controls and pat. with low temp. on 3rd day		–		< 0.05		< 0.01

and it may be noted that the pressures were nearly the same in the two groups

The influence of time may be partly separated from that of temparature by excluding patients with temperature of 38 C or higher when comparing values of the 1st and 3rd day On the 1st day 9 patients had a temperature less than 38 C and the same applied to 7 patients on the 3rd day On the 1st and 3rd day the mean heart rates of those patients were 76 and 80 beats/min the cardiac outputs 5 4 and 4 7 l/min. stroke volumes 74 and 60 ml arterial blood pressures were 127/68 mean 92 and 111/59 mean 81 mm Hg and resistances 18 and 18 units. Although the values for cardiac output, stroke volume and arterial pressure seemed much lower on the 3rd day there was no significant difference

Of the nine patients with a temperature less than 38 C on the 1st day in six the temperature subsequently rose above 38° C while three still had a low temperature the following days. There was no difference in mean haemodynamic data on the 1st day between these six and these three patients. All but one patient with a low temperature on 1st day of infarction had cardiac output determined also on the 2nd or 3rd day In six patients there had been a rise in temperature before the second determination Three of them had a rise in output, two an unchanged output and in one patient there was a lower output. In two patients the temperature was low also at the second cardiac output determination and the output was unchanged

The comparisons of circulatory data described above are collated in table III The mean age of the groups was similar Significant differences between controls and patients with a temperature under 38 C on the 3rd day are included The patients had a lower cardiac output, stroke volume and brachial arterial pressure than the controls

Forty-eight observations were available for multivariate regression analysis of cardiac output as a function of time and temperature The output is almost significantly ($p = 0.12$) correlated to time and significantly ($p < 0.01$) to change in temperature ($R = +0.40$) The estimated function is

$$CO = -17.7 - 0.3\,d + 0.63\,t$$

where CO = cardiac output (l/min.) d

on these days and of patients with temperature 38° C or higher on 3rd day (means and SE of means)

Brachial arterial pressure (mm Hg)						Resistance (units)		Left ventricular work kpm/min)		Left ventricular stroke work(pm)	
Systolic		Diastolic		Mean							
1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd
127±7	111 ± 5	68 ± 5	59 ± 4	97 ± 6	81 ± 4	18 ± 1	18 ± 1	6.8±0.6	5.2±0.5	92 ± 7	68 ± 8
	109 ± 3		58 ± 2		78 ± 3		14 ± 1		6.0±0.3		63 ± 6
	–		–		–		< 0.05		–		–
	< 0.01		< 0.05		< 0.02		–		< 0.01		< 0.001

= day of infarction (1st, 2nd or 3rd) t = temperature (°C in the morning)

Cardiac output fell about 0.3 l/min per day over the first three days, and rose about 0.6 l/min. per degree rise in temperature.

Regression analysis of resistance and day of infarction did not show any significant correlation. Resistance and morning temperature were significantly correlated ($p < 0.02$) (= — 0.35) The estimated function is

R = 75 4—1.57 t

where R = resistance (arbitr units) t = temperature (C in the morning)

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When the systolic pressure during the first 3 days of myocardial infarction was above 120 mm Hg the resistance was

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(The number of patients in the groups were 9 7 and 11)

Temp	Heart rate (beats/min)		Cardiac output (l/min)		Stroke vol. (ml)	
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≧ 38 C		91 ± 6		5.6 ± 0.3		65 ± 5
Probabil. of diff between high and low temp. on 3rd day		–		< 0.05		–
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and it may be noted that the pressures were nearly the same in the two groups.

The influence of time may be partly separated from that of temparature by excluding patients with temperature of 38 C or higher when comparing values of the 1st and 3rd day On the 1st day 9 patients had a temperature less than 38° C and the same applied to 7 patients on the 3rd day On the 1st and 3rd day the mean heart rates of those patients were 76 and 80 beats/min the cardiac outputs 5 4 and 4 7 l/min stroke volumes 74 and 60 ml, arterial blood pressures were 127/68 mean 92 and 111/59 mean 81 mm Hg and resistances 18 and 18 units. Although the values for cardiac output stroke volume and arterial pressure seemed much lower on the 3rd day there was no significant difference

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Forty-eight observations were available for multivariate regression analysis of cardiac output as a function of time and temperature The output is almost significantly ($p = 0\ 12$) correlated to time and significantly ($p < 0\ 01$) to change in temperature ($R = +0\ 40$) The estimated function is

$$CO = -17\ 7 - 0\ 3\ d + 0.63\ t$$

where CO = cardiac output (l/min) d

Patients with arrhythmia

One male patient had a total atrioventricular heart block on the 1st day He had a heart rate of 47 beats/min., cardiac output 4.2 l/min. and arterial blood pressure 110/50 mean 65 mm Hg After metaraminol infusion he changed to sinus rhythm as described elsewhere (9)

One female patient who had mitral insufficiency of rheumatic origin already had atrial fibrillation before the infarction. She was investigated on the 2nd day of infarction. Her heart rate was 53 beats/min., cardiac output 2.6 l/min. and arterial blood pressure 120/40 mean 60, mm Hg

Patients dying in hospital

Seven patients died in hospital, one on the 3rd day one on the 4th, two on the 10th and one each on the 17th, 19th and 34th day All were men and all had sinus rhythm during haemodynamic investigations. All but two had a morning temperature above 38° C on at least one day of investigation. The mean heart rate on day of higher temperature, 1st day in one patient, 2nd day in 4 patients and 3rd day in 2 patients, was 92 The corresponding cardiac output was 6.0 l/min. stroke volume 63 ml, brachial arterial pressures 115/68, mean 88, mm Hg and resistance 15 units.

These means did not differ significantly from the group means. The lowest cardiac output encountered in the group was 4.2 l/min. nd the lowest brachial arterial pressures were 107/70, mean 81 mm Hg

Two patients who subsequently died had cardiac output determined on the 1st and the 2nd day of infarction. Both showed a considerable rise in temperature between those days. Both had high cardiac outputs the first as well as the second day There was a moderate fall in brachial arterial mean pressure and corresponding fall in resistance.

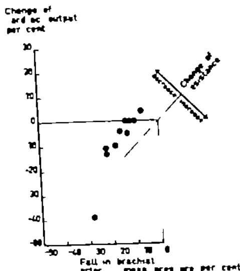


Fig 5. Percentage changes in cardiac output and changes in resistance in patients with fall in brachial arterial mean pressure in the first days following infarction.

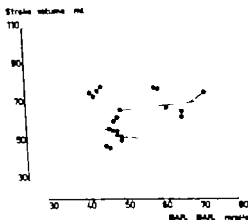


Fig. 6. Stroke volume in relation to pulse pressure (BAPs—BAPD) in male patients during the first three days of myocardial infarction. Symbols belonging to the same patient are connected by lines.

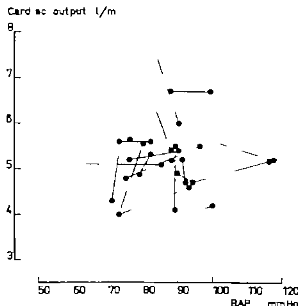


Fig 3. Cardiac output in relation to brachial arterial mean pressure (BAPm) in male patients during the first three days of myocardial infarction. Symbols belonging to the same patient are connected by lines.

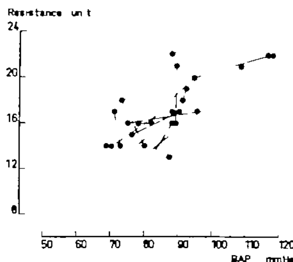


Fig 4 Peripheral vascular resistance in relation to brachial arterial mean pressure in male patients during the first three days of myocardial infarction. Symbols belonging to the same patient are connected by lines.

19 ± 1 and when it was below 110 mm Hg the resistance was 15 ± 1 units The difference between these values is significant ($p < 0.001$) The relation between brachial arterial systolic pressure and resistance was thus good.

The relation of changes in brachial arterial systolic pressure to changes in peripheral resistance was similar to that in fig 4

When the pulse pressure in male patients with sinus rhythm during the first 3 days of myocardial infarction was above 55 mm Hg the stroke volume was 70 ± 3 and when the pressure was below 45 mm Hg the volume was 65 ± 7 There was no significant difference between these values. There was nevertheless a significant ($p < 0.05$) correlation between pulse pressure and stroke volume ($r = +0.33$) The values are graphed in fig 6

The relation of changes in pulse pressure to changes in stroke volume from day to day was poor in the individual patients, as seen in the same figure.

Females

One of the female patients with sinus rhythm was 69 years old and was investigated on the 3rd day of myocardial infarction. The other was 64 years and was investigated on the 1st, 2nd and 3rd days. The 69 year-old patient had a morning temperature of 38 0 C on the day of investigation Her heart rate was 83 beats/min cardiac output 3 6 l/min. brachial arterial blood pressure 120/64 mean 89 mm Hg and resistance 25 units The other female patient had morning temperatures of 37 0 37 9 and 38.5 C Her heart rate was 60 beats/min. on the first two days and 73 on the third day Cardiac output was 4 0 l/min each day Brachial arterial blood pressure fell from 128/65 mean 93 mm Hg on the 1st day to 93/59 mean 74 mm Hg on the 3rd day The corresponding resistances were 24 and 18 units.

rhythm on the 16th to 43rd day of myocardial infarction (For calculation of difference between recumbent and

Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
	Systolic	Diastolic	Mean			
14 43 ± 1 5	16 132 ± 5 20	16 73 ± 3 10	16 96 ± 3 13	16 18 ± 1 3	16 7.0 ± 0.4 1.7	16 99 ± 6 24
16 31 ± 2 6	16 122 ± 3 13	16 67 ± 1 6	16 88 ± 2 8	16 19 ± 1 3	16 5.8 ± 0.4 1.5	16 84 ± 6 23
14 +9 ± 1	14 −5 ± 2	14 −3 ± 1	14 −4 ± 1	14 +2 ± 0	14 −1.1 ± 0.2	14 −15 ± 3
< 0.001	< 0.05	< 0.05	< 0.01	< 0.001	< 0.001	< 0.001

myocardial infarction in male and female patients with sinus rhythm

Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
Systolic	Diastolic	Mean			
21 −8.3 ± 3.9 17.6	21 −3.3 ± 2.4 11.1	21 −7.5 ± 2.9 13.3	21 −1.9 ± 0.7 3.2	21 −0.31 ± 0.28 1.28	21 −7.1 ± 3.4 15.6
< 0.05	—	< 0.02	< 0.05	—	
13 +18.2 ± 6.0 21.9	13 −13.1 ± 2.8 9.9	13 −14.5 ± 3.3 11.8	13 +1.5 ± 0.9 3.2	13 +1.22 ± 0.46 1.65	13 +22.8 ± 3.9 21.5
< 0.02	< 0.001	< 0.001	—	< 0.05	< 0.01

Table IV Mean values of haemodynamic data at rest, recumbent and sitting of male patients with sinus sitting female patients with sinus rhythm are included)

Body position	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)	Oxygen consumption (ml/min)
Recumbent				
No.	16	16	16	14
Mean ± SE of mean	72 ± 4	5.3 ± 0.2	76 ± 4	224 ± 7
SD	14	0.9	16	27
Sitting				
No.	16	16	16	16
Mean ± SE of mean	71 ± 3	4.8 ± 0.2	70 ± 4	240 ± 7
SD	13	0.9	16	28
Diff between recumbent and sitting				
No.	14	14	14	14
Mean ± SE of diff.	±0 ± 1	−0.6 ± 0.1	−9 ± 2	+11 ± 3
Probabil. of diff.	–	< 0.001	< 0.001	< 0.01

Table V Mean values of changes in haemodynamic data between 2nd and 1st day and 3rd and 2nd day of

Compared	Heart rate (beats/min)	Cardia output (l/mm)	Stroke vol. (ml)
2nd and 1st day and 3rd and 2nd day			
No.	21	21	21
Mean ± SE of mean	+3.0 ± 2.4	+0.20 ± 0.17	−0.9 ± 1.9
SD	11.2	0.77	8.6
Probabil. of diff.	–	–	–
16th to 43rd day with 3 d 2nd or 4th day			
No.	13	13	13
Mean ± SE of mean	−8.5 ± 4.2	+0.27 ± 0.25	+9.3 ± 4.3
SD	15.0	0.92	16.3
Probabil. of diff.	–	–	–

before leaving hospital, heart rate cardiac output and resistance were similar while the stroke volume systolic, diastolic and mean pressures, left ventricular work and left ventricular stroke work had increased.

The cardiac output and resistance in male patients with sinus rhythm investigated on the 1st, 2nd or 3rd days and 16th to 43rd day are depicted in figs. 7 and 8. Patients with a temperature less than 37° C are symbolized by open circles, patients with a temperature from 37° C to 38° C by semi filled circles and patients with a higher temperature by filled circles. Symbols for the same patient are connected by thin lines.

In 13 patients observations made on the 3rd, 2nd or 4th day could be compared with observations before they left hospital. The significant changes in those paired observations were rise in systolic, diastolic and mean arterial pressures and rise in left ventricular work and left ventricular stroke work (table V)

Complications

In three patients the pulsations of the radial artery disappeared during the investigation or were not felt after the catheters were withdrawn. These patients were treated with warmth around the arm, heparin and papaverin HCl. The pulsations reappeared within some hours.

One patient developed thrombosis at the puncture site in the ulnar artery which was followed by necrosis in the fingertips. He was 37 years old and experienced his second infarction. He died suddenly on the 31th day. Autopsy showed total occlusion of the left coronary artery in two places and extensive infarction of anterior and posterior walls and septum, with aneurysm and thrombus formation. The puncture had been in the ulnar artery and a thrombus had formed at the puncture site. There was arteriosclerosis in ulnar and other arteries of the arm.

Discussion

Mean cardiac outputs on each of the first three days of infarction were virtually the same and a similar mean cardiac output was found prior to discharge from hospital on the 16th to 43rd day of infarction. But this picture changed considerably when the temperature of the patients was taken into consideration. Cardiac output seems generally to have decreased in the patients whose temperature did not rise to 38° C on the 3rd day. It remained essentially unchanged in the patients who experienced a rise in temperature to or above 38 C. These changes were not statistically significant. A significant difference was, however obtained when the 3rd day cardiac output values were analysed separately. Patients with a temperature below 38° C had a lower cardiac output than those with a higher temperature. Hence infarction uncomplicated by a marked temperature rise seems accompanied by a decreased cardiac output on the 3rd day of infarction. This fall in cardiac output may be due to infarction or to bed rest. The fact that all patients who had cardiac output below 5 l/min. on the 3rd day of infarction showed a more or less marked increase at the end of the hospital stay suggests that myocardial infarction caused the cardiac output decrease during the acute stage. A body-temperature rise appears to counteract the tendency to a cardiac output decrease on the 3rd day of infarction.

Concerning brachial arterial pressures there was a decrease with time in systolic

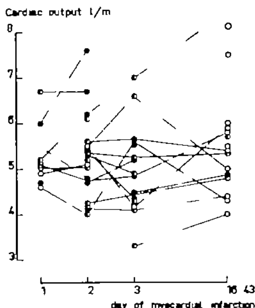


Fig 7 Cardiac output in male patients during the first three days of myocardial infarction and before leaving hospital. Morning temperature below 37° C is indicated by unfilled circles, temperature from 37° C to 38° C by semi-filled circles and temperature f 38 C or higher by filled circles. Symbols belonging t the same patient are connected by lines.

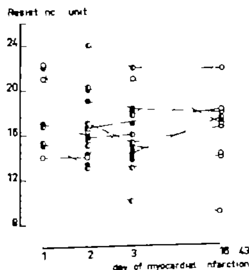


Fig 8. Peripheral vascular resistance in male patients during the first three days of myocardial infarction and before leaving hospital. Same symbols as in fig 7

SUBACUTE STAGE

The investigations were repeated once more in 14 male patients, and in 4 patients the first investigation was made between the 16th and 43rd day in hospital.

Five patients had a morning temperature between 37 0 and 37 3 C on the day of investigation prior to discharge. The others had a temperature below 37° C.

Mean haemodynamic data are given in table V One patient with a cardiac output of 11 2 l/min. recumbent is, as mentioned excluded from the calculations

Mean oxygen consumption increased from 224 ml/min when the patients were recumbent to 240 ml/min. when they were sitting ($p < 0\ 01$) Heart rates were 72 and 71 respectively Cardiac output decreased from 5.3 to 4 8 l/min. ($p < 0\ 001$) and stroke volume from 76 to 70 ml ($p < 0\ 001$) when the patients changed position. Arteriovenous oxygen difference increased from 43 to 51 ml/l ($p < 0\ 001$) Brachial arterial systolic pressures decreased from 132 to 122 mm Hg diastolic pressures from 73 to 67 and mean pressures from 96 to 88 mm Hg

Mean total peripheral resistance increased from 18 units recumbent to 19 units sitting ($p < 0\ 001$) Left ventricular work and stroke work both decreased ($p < 0\ 001$) There were no differences in recumbent circulatory data between controls and patients prior to discharge

Subacute and acute stages compared

When means from the 1st day of myocardial infarction were compared with those for patients investigated before discharge, no significant differences were noted When means for patients with a temperature less than 38 C on the 3rd day of myocardial infarction were compared with those for patients investigated

cute stage studies						Follow-up studies					
Stroke vol. (ml)	Blood pressure (mm Hg)			Resistance (authors' units)	No. of pat. (died)	Time after onset of infarction (weeks)	No. of pat.	Cardiac output (l/min)	Cardiac index	Stroke vol. (ml)	Resistance (authors' units)
	Systolic	Diastolic	Mean								
—	102	70	—	—	0	3	4	Same in 3; +50% in one	—	—	—
76	—	—	93	15	0	—	—	—	—	—	
50	—	—	89	17	1						
27	—	—	79	26	3						
—	—	—	86	18	0	6—8	9	—	2.8	—	19
—	—	—	70	23	6						
53	139	73	106	20	2	—	—	—	—	—	—
32	133	81	101	30	4						
21	85	59	73	38	7						
104	—	—	—	16	2	4	4	—0.2	—	+ 3	— 4
72	—	—	—	20	1		5	±0	—	+ 2	— 2
53	—	—	—	22	4		7	+1.7	—	+34	— 7
75	132	81	98	16	2	4—8	9	7 7	4.1	88	14
70	—	—	—	—	0	3—4	18	+0.4	+0.2	+ 7	—
43	—	—	—	—	8		9	+1.5	+0.8	+22	—

same patients. A significant rise of the blood pressure t the end of hospital stay seems to be due to small and not significant increases in cardiac output, especially in patients with a temperature below 38° C, and in peripheral resistance. The blood pressure fall during the acute stage may thus be the combined result of a cardiac factor — impaired myocardial function — and a peripheral factor — peripheral vasodilatation. The contribution of the peripheral factor seems to be determined by body temperature the higher the temperature the lower the resistance

The influence of temperature on cir culation in patients with infarction is probably the result of a metabolite effect. Fever induced by pyrogens (5) influences the normal circulation similarly to fever accompanying myocardial infarction. Increase of cardiac output and decrease of

Table VI Circulatory studies of patients in acute myocardial infarction

Year	Author and method used	Acute stage studies				
		Time after onset of Infarction	Clinical grouping	No. of pat.	Cardiac output (l/min)	Cardiac index
1950	Pritchard & Hellerstein (10) Atrial catheterization	Within 10 days	Mild	6	—	2.0
1952	Freis et al. (2) Indicator dilution technique with sampling of arterial blood	12–72 hours	Mild	4	6.6	3.4
			Severe	4	5.3	2.9
			Shock	3	3.2	1.8
1954	Smith et al. (12) Indicator dilution technique with sampling of arterial blood	Within 24 hours	Mild	9	—	2.4
			Shock	7	—	1.6
1954	Gilbert et al. (4): Indicator dilution technique with sampling of arterial blood	6–48 hours	Mild	7	4.8	2.6
			Severe	6	3.3	1.9
			Shock	7	1.8	1.0
1955	Gammill et al. (3) Indicator dilution technique with sampling of arterial blood	Within 39 hours	Mild	11	7.7	4.3
			Severe	12	5.7	3.2
			Shock	14	5.3	2.9
1957	Lee (7) Indicator dilution technique with sampling of arterial blood	4–99 hours	Mild and severe	11	6.7	3.7
1959	Broch et al. (1) Indicator dilution technique with oxymetry	6–99 hours	Mild	18	5.6	3.0
			Severe and shock	17	3.6	[illegible]

and mean pressures and a tendency to decreased diastolic pressure. The temperature did not seem to affect this decrease. Bed rest did not seem responsible for the decreased pressure during the acute stage because blood pressure was significantly higher at the end of the hospital stay This is also in accordance with general clinical experience.

Calculated peripheral resistances from all investigations during the first three days of infarction were closely related to blood pressure (fig 4) An influence of body temperature was, however present here too patients with a higher temperature had a significantly lower peripheral resistance on the 3rd day of infarction than patients with a lower temperature. Peripheral resistance tended to be increased at the end of the hospital stay compared with the measurements during 2nd 3rd or 4th day of infarction in the

Table VI illustrates great differences between haemodynamic data in patients with acute myocardial infarction obtained by different authors. Many factors may be responsible for these differences. The results emphasize the importance of control values in judging the significance of observed pathological changes. Moreover the lack of information in the literature concerning body temperature invalidates comparison of the present data with published data.

Summary

Haemodynamic data have been recorded during the first three days of myocardial infarction and again before the patients left hospital.

During the first few days the cardiac output decreased and the brachial arterial pressures fell. In patients with fever there was a fall in peripheral resistance but not in output, similarly leading to lower brachial arterial pressures.

Before discharge these patients parameters reached normal levels.

Our data suggest that acute myocardial infarction as a disease interferes both with heart performance and with regulation of the peripheral resistance. Body temperature has a marked influence on the regulation of the peripheral resistance and is haemodynamically an important factor in the course of the disease.

Acknowledgement

This study was supported by a grant from the Swedish National Association against Heart and Lung Diseases.

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peripheral resistance were, however more pronounced with normal circulation. It is hard to tell whether these differences are due to the state of circulation or are attributable to the age differences between the normals and the patients with myocardial infarction.

Stroke volume showed a general tendency to decrease to the 3rd day of infarction and to increase again to the end of hospital stay Heart rate usually varied in the opposite direction which may have prevented an excessive decrease in blood flow per minute.

Comparisons of haemodynamic data in patients and in controls disclosed that stroke volume was lower in patients already on the 1st day of infarction. This difference was more marked when comparison was made with stroke volumes for patients with a body temperature below 38 C on the 3rd day of infarction On 3rd day of infarction cardiac output and blood pressure were lower in these patients than in the controls. Peripheral vascular resistance was similar in the patients and controls. This supports the above suggestion that reduced myocardial function is responsible for the acute haemodynamic deterioration It demonstrates furthermore that peripheral resistance is not lowered by infarction unless the temperature rises.

Comparison of the data recorded at the end of the hospital stay and values for controls showed that the patients regained their normal haemodynamic equilibrium at rest in a rather short time. It shows furthermore that prolonged bed rest and hospital stay did not decisively influence the values recorded in patients.

Left ventricular work in patients followed essentially the general haemodynamic pattern throughout the stages of the infarction. Left ventricular work, which appeared to be somewhat lower already on the 1st day of infarction than in controls, was significantly lower on the 3rd day The decrease was especially pronounced in patients with a temperature below 38 C. Left ventricular work rose above the 1st-day values prior to discharge, approaching values for the controls.

Changes in left ventricular stroke work showed the same general pattern as those of left ventricular work but were more pronounced. Left ventricular stroke work had dropped considerably more than left ventricular work by the 3rd day Values for left ventricular stroke work at the end of the hospital stay approach these of the controls and were higher than on the 1st day of infarction The behaviour of left ventricular stroke work throughout the course of infarction is in accordance with the above suggestion that infarction interferes to a large extent with the myocardial function of the left ventricle.

Nine studies were performed on seven patients who died in hospital. Five of these patients had a temperature above 38 C on at least one day of investigation. Mean haemodynamic data on the day of higher temperature were essentially the same as in the whole group of comparable patients. The course of the disease could not be predicted from these haemodynamic data, which contrasts with the findings of Broch et al (1)

When patients changed from a recumbent position to a sitting position in the investigation prior to discharge from hospital there was a fall in brachial pressures in contrast to no change in normal subjects, about 30 years of age (8) There was thus a change in the adaptation of the circulation to sitting but the comparison is invalidated by the considerably younger age of the control subjects.

Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Haemodynamics During Rest and Exercise at the End of Convalescence from Myocardial Infarction

Comparison with Earlier and Later Stages of the Disease

By

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Haemodynamic studies of patients with acute myocardial infarction have been followed by investigations on the same patients up to two months after the infarction (1 3 4 7—10) The circulation has then been found to have returned towards normal. No tests have been used to detect possible small abnormalities of the circulation.

Patients investigated by the authors in the acute stage and in early convalescence have been examined again before their return to work. The haemodynamic follow-up period was thereby extended to some months after the infarction and a standardized exercise test was used in the haemodynamic study

Comparisons are made with haemodynamic data recorded by the same technique in the majority of the same patients in the acute stage and in early convalescence (7) and with haemodynamic data recorded by the same technique in another patient material 1—11 years after myocardial infarction (5)

Submitted for publication June 11 1963

Material

All 19 patients were males. They were investigated in the 3rd to 5th, in one instance the 7th and in another the 13th month after myocardial infarction. Age range was 39 to 63 mean 53 years.

All had been investigated in the acute stage or early convalescence (7)

During the acute stage they had clinical and electrocardiographic signs of myocardial infarction. All but three had a temperature reaction. The S-GOT rose to values between 75 and 460 Sixteen had ST segment elevation and development of pathological Q waves while one each had only ST segment elevation or development of pathological Q waves or T wave inversions. Nine had predominantly anterior infarction and 9 predominantly posterior infarction while one had an infarction electrocardiographically extending over both surfaces. Five had brachial arterial systolic pressure of 90 mm Hg or less during some part of the acute stage. Two patients had heart size greater than 450 ml/sqm body surface area before they left hospital and in four there were signs of pulmonary stasis at the same time.

During stay at home seven patients had complained of effort angina. There were symptoms or signs of heart failure at reinvestigation in five patients. Nitro-preparations

myocardial infarction

Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (gm)
	Systolic	Diastolic	Mean			
18 48 ± 2 9	18 130 ± 4 16	18 74 ± 1 6	18 97 ± 2 10	18 19 ± 1 3	18 6.9 ± 0.3 1.2	18 103 ± 5 20
19 55 ± 1 6	19 130 ± 4 16	19 74 ± 2 10	19 96 ± 3 11	19 21 ± 1 3	19 6.0 ± 0.2 0.9	19 87 ± 3 13
17 108 ± 2 10	17 157 ± 5 22	17 80 ± 3 12	17 109 ± 4 15	17 14 ± 1 2	17 11.9 ± 0.4 1.7	17 122 ± 6 25
18 +8 ± 1 < 0.001	18 −2 ± 1 –	18 −2 ± 1 –	18 −4 ± 1 < 0.02	18 +2 ± 1 < 0.01	18 −1.1 ± 0.2 < 0.001	18 −19 ± 4 < 0.001
17 +51 ± 2 < 0.001	17 +27 ± 2 < 0.001	17 +6 ± 2 < 0.01	17 +13 ± 2 < 0.001	17 −7 ± 1 < 0.001	17 +5.8 ± 0.3 < 0.001	17 +32 ± 5 < 0.001

The exercise test was performed sitting on bicycle ergometer

The methods have been described in detail in other papers (5 6)

Results

Rest, recumbent and sitting

Mean oxygen consumption recumbent was 246 ml/min. and 253 ml/min. sitting The heart rates were 67 and 70 beats/min. respectively

Mean cardiac outputs were 5.3 and 4.6 l/min. recumbent and sitting ($p < 0.001$) mean stroke volumes were 80 and 67 ml ($p < 0.001$) and mean arteriovenous oxygen differences 48 and 55 ml/l ($p < 0.001$)

Mean systolic brachial arterial pressure was 130 mm Hg recumbent and sitting mean diastolic pressure was 74 mm Hg and the mean of mean pressures was 97 and 96 mm Hg respectively ($p < 0.02$)

Mean total peripheral resistance was 19 and 21 units recumbent and sitting ($p < 0.01$) Left ventricular work was

Table I Means of haemodynamic data for male patients with sinus rhythm 3 to 13 months after

Body position (work load)	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)	Oxygen consumption (ml/min)
Recumbent				
No.	18	18	18	18
Mean ± SE of mean	67 ± 2	5.3 ± 0.2	80 ± 4	246 ± 7
SD	8	0.8	17	29
Sitting				
No.	19	19	19	19
Mean ± SE of mean	70 ± 2	4.6 ± 0.1	67 ± 2	253 ± 6
SD	9	0.5	10	26
Sitting (200 kpm/min)				
No.	17	17	17	17
Mean ± SE of mean	100 ± 4	8.0 ± 0.2	82 ± 4	843 ± 16
SD	16	0.8	17	66
Diff between recumbent and sitting				
No.	18	18	18	18
Mean of diff. ± SE of means of diff.	+2 ± 1	−0.7 ± 0.1	−12 ± 3	+6 ± 4
Probabil. of diff.	—	< 0.001	< 0.001	—
Diff between sitting at rest and working (200 kpm/min)				
No.	17	17	17	17
Mean of diff. ± SE of means of diff.	+31 ± 3	+3.3 ± 0.2	+14 ± 3	+590 ± 13
Probabil. of diff.	< 0.001	< 0.001	< 0.01	< 0.001

were used by eight and digitalis by three patients.

All patients had sinus rhythm. The ECGs of two patients were completely normalized. In 16 patients there were residual pathological Q and T waves and in one patient pathological T waves were the only remaining signs of the infarction.

Exercise tolerance tests for evaluation of electrocardiographic response to work were performed at reinvestigation. ECGs during and after work were analysed as described by Söderholm et al. (11). There was a pathological change of the ECG in six patients.[1]

Fourteen healthy controls, aged 44 to 58 mean 51 years were investigated by the same technique sitting at rest (6). Ten of these men performed a 200 kpm/min. exercise test on a bicycle ergometer. Their results are used for comparison with the results of the exercise test performed by the patients.

Methods

Catheters were inserted percutaneously and intraarterial pressures recorded. Cardiac output was determined by dye dilution technique

[1] The electrocardiograms of rest and exercise were recorded in the Department of Clinical Physiology (Head Prof. A. Carlsten, M. D.) and analysed together with Assistant Prof. B. Söderholm, M. D.

for controls (Mean values and SE of means are given)

Oxygen consumption (ml/min)	Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
		Systolic	Diastolic	Mean			
—	—	139 ± 7	71 ± 3	98 ± 4	18 ± 1	7.7 ± 0.6	114 ± 7
—	—	127 ± 7	68 ± 5	92 ± 6	18 ± 1	6.8 ± 0.6	92 ± 7
—	—	111 ± 5	59 ± 4	81 ± 4	18 ± 1	5.2 ± 0.5	68 ± 8
224 ± 7	43 ± 1	132 ± 5	73 ± 3	96 ± 3	18 ± 1	7.0 ± 0.4	99 ± 6
246 ± 7	48 ± 2	130 ± 4	74 ± 1	97 ± 2	19 ± 1	6.9 ± 0.3	105 ± 5

infarction were similar in age to the present patients at the time of study but significantly younger at the time of their infarction, when the former were 41 to 58 mean 47 years old and the latter were 39 to 63 mean 53 years old. The ischaemic heart disease may be more extensive and aetiologically different and more progressive in the younger patients. This could influence differences between the two series. Patients from both series, however returned to their previous work after a convalescence period

Patients who were unable to return to work were older than the present patients at the time of study but of similar age at the time of infarction.

Rest recumbent

All haemodynamic data recorded at rest recumbent in the present investigation were of the same order as in the control subjects (7) whose mean age was 4 years higher Oxygen consumption and arteriovenous oxygen difference were higher in the patients of the present investigation than in the patients investigated at the end of the hospital stay The mean age of the latter was 7 years higher A return of haemodynamic data towards normal takes place during the first month after infarction (7) Some parameters, however soon again seem to be abnormal.

Rest sitting

Cardiac output was lower than in controls of similar age and in patients 1—7 years after infarction who had returned to work, but was of the same order as in patients at end of the hospital stay and in patients who had been unable to return to work 3—11 years after infarction.

There were no significant differences in stroke volume between the end of the hospital stay and the present study or between the present study and values from patients 3—11 years after infarction who did not return to work. Stroke volume in controls and in patients who had returned to work 1—7 years after infarction was significantly higher than in patients of the present study, suggesting that stroke volume may increase with time in patients returning to work and does not do so in patients who are unable to work.

Arteriovenous oxygen difference was higher in the patients of the present investigation than in the controls. It was also higher than in the patients investigated before leaving hospital and in patients who had returned to work. Those who were unable to work showed a similarly high

Table II Haemodynamic data at rest recumbent, for patients in earlier stages of myocardial infarction and

Myocardial infarction	Age (yrs)	No. of pat.	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)
Controls	57	12	68 ± 2	5.7 ± 0.2	85 ± 3
1st day temp. < 38	58	9	76 ± 7	5.4 ± 0.2	74 ± 5
3rd day temp. < 38	60	7	80 ± 6	4 7 ± 0.4	60 ± 6
16th to 43rd day	60	16	72 ± 4	5.3 ± 0.2	76 ± 4
3rd to 13th month	53	18	67 ± 2	5.3 ± 0.2	80 ± 4

6 9 and 6 0 kpm/min. ($p < 0\ 001$) and left ventricular stroke work was 105 and 87 pm ($p < 0\ 001$)

200 kpm/min exercise test on bicycle ergometer

Seventeen patients performed the exercise test. Mean oxygen consumption was 843 ml/min. during exercise and mean heart rate was 100 beats/min Mean cardiac output was 8 0 l/min stroke volume 82 ml and arteriovenous oxygen difference 106 ml/l. Mean brachial arterial systolic pressure was 157 mm Hg Diastolic and mean pressures were 80 and 109 mm Hg respectively Mean total peripheral resistance was 14 units. Left ventricular work was 11 9 kpm/min and left ventricular stroke work was 122 pm.

All values during work differed significantly from those at rest.

Means at rest recumbent and sitting and in the exercise test, and differences between circulatory data recumbent and sitting and between rest and exercise, are tabulated in table I

The calculated heart rate increase per 100 ml incremental oxygen consumption was 5.2 ± 0 5 beats/min The calculated increase in cardiac output was 0 57 ± 0 03 l/min. and in systolic arterial pressure 4 7 ± 0 5 *mm Hg* per 100 ml incremental oxygen consumption during the exercise. The corresponding values for diastolic and mean pressure increases were 1 0 ± 0 3 and 2 3 ± 0 3 mm Hg

Discussion

Haemodynamic data for the patients now investigated can be compared with data for the majority of the same patients *obtained in the acute phase of infarction* and at the end of the hospital stay (7) (table II fig 1) Comparison can also be extended to cover the data of other patients investigated 1—7 mean 4 years after infarction who had returned to work and of patients investigated 3—11 mean 7 years after infarction who had not been able to retain their work. In making this comparison it should be kept in mind that all the present patients returned to their previous occupation after a convalescence period (table III fig 2) Such comparison may provide valuable information concerning the haemodynamic course from acute phase to several years of the disease. Homogeneity of the series is then important. Patients studied 1—7 years after

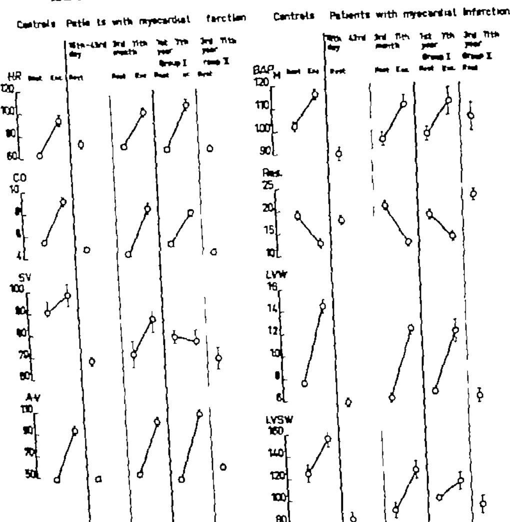


Fig. 2. Means and their standard errors of haemodynamic data for controls aged 44 to 58, mean 51 years and for patients with myocardial infarction. All investigations were performed sitting. Group I patients who had returned to work after their myocardial infarction. Group II patients who had not been able to return to gainful activity after their myocardial infarction.

Exc: exercise on bicycle ergometer 200 kpm/min.
HR heart rate, beats/min.
CO- cardiac output, l/min.
SV stroke volume ml.
A-V arteriovenous oxygen difference, ml/l.
BAPm brachial arterial mean pressure, mm Hg
Res. peripheral vascular resistance, units.
LVW left ventricular work, kpm/min.
LVSW left ventricular stroke work, gm.

tween controls and patients or between patients in different phases of infarction. In the presence of decreased cardiac output the higher heart rate may be regarded as a sign of circulatory insufficiency rather than due to some stress factor caused by the investigative procedure.

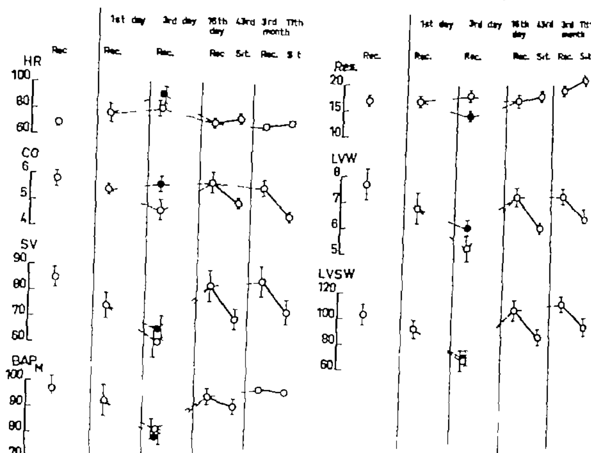


Fig. 1 Means and their standard errors of haemodynamic data for controls aged 50 to 67 mean 57 years and for patients with myocardial infarction. On the third day the means for patients with temperature 38 or over were separated from other patients values and symbolized by filled circles. Before leaving hospital and at end of convalescence patients were investigated recumbent (Rec.) and sitting (Sit.)

HR	heart rate, beats/min.	Res.	peripheral vascular resistance, units
CO	cardiac output, l/min.	LVW	left ventricular work, kpm/min.
SV	stroke volume, ml.	LVSW	left ventricular stroke work, pm.
BAPM	brachial arterial mean pressure mm Hg		

arteriovenous oxygen difference. The changes thus rather strictly followed the pattern of changes in cardiac output. The differences between controls and patients before leaving hospital and patients of the present study when investigated recumbent were born out by differences at investigations sitting

Total peripheral resistance during various phases of infarction was not significantly different from that in controls, except for patients who were unable to return to work and had higher peripheral resistance.

Mean brachial arterial blood pressure which was lower on the 3rd day of hospital stay than in controls, showed a clear tendency to increase with time. It was highest in patients who were unable to return to work, in whom it reached the mean for controls.

Heart rate was higher on the 3rd day of infarction than in the present study. No further differences were found either be

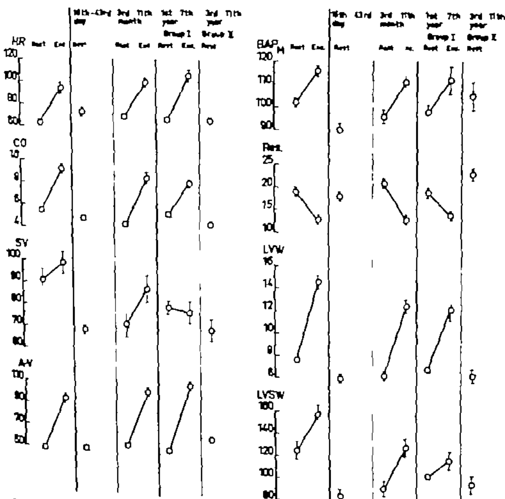


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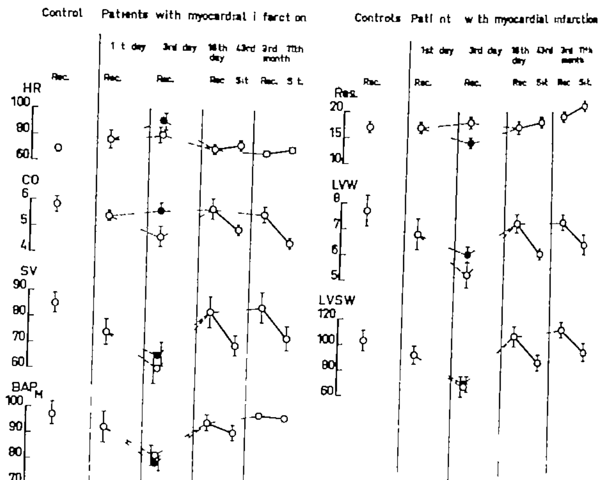


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controls (Mean values and SE of means are given)

Oxygen consumption (ml/min)	Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
		Systolic	Diastolic	Mean			
265 ± 6	49 ± 2	139 ± 4	81 ± 2	102 ± 2	19 ± 1	7.6 ± 0.3	125 ± 8
240 ± 7	51 ± 2	122 ± 3	67 ± 1	88 ± 2	19 ± 1	5.8 ± 0.4	84 ± 6
253 ± 6	55 ± 1	130 ± 4	74 ± 2	96 ± 3	21 ± 1	6.0 ± 0.2	87 ± 5
258 ± 10	50 ± 2	133 ± 3	76 ± 3	98 ± 3	19 ± 1	7.1 ± 0.5	105 ± 4
273 ± 16	61 ± 3	145 ± 10	81 ± 5	105 ± 6	23 ± 1	6.6 ± 0.6	99 ± 8

at rest on the 16th to 43rd day of myocardial infarction and 3 to 13 months after falling ill

Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
	Systolic	Diastolic	Mean			
11	11	11	11	11	11	11
+4.7 ± 2.4	+9 ± 3	+7 ± 2	+7 ± 3	+2.9 ± 0.8	−0.1 ± 0.6	+0.1 ± 6.1
0.9	11	7	9	2.7	2.0	20.2
–	< 0.02	< 0.02	< 0.05	< 0.01	–	–

myocardial disease neuro-humoral factors or muscular inactivity during protracted hospital stay Thus changing of body posi tion may be regarded as a test of those factors

The decrease seen in cardiac output, stroke volume, left ventricular work and left ventricular stroke work and mean ar terial pressure, the slight increase in ar teriovenous oxygen difference and periph eral vascular resistance and the unaltered heart rate were changes similar to those seen in the study of the patients (7) before they left hospital. Blood pressure showed a slight tendency to decrease in the patients but not in the controls (6) This tend ency may be ttributed to the appre ciable age difference between patients and control subjects. Thus, this relatively simple test did not disclose any conclusive evidence of gross disturbance in circula tory regulation in patients after infarction and prolonged hospital stay compared with relatively young active controls.

Effect f exercise

The trends of difference between pa tients and controls at rest could, among other factors, be due either to the fact that the methods employed are not sensitive enough to disclose real differ ences caused by the disease or that the circulation is in normal haemodynamic equilibrium. A standardized exercise test may thus help to render significant differences not significant at rest.

Heart rate t work load 200 kpm/min on bicycle ergometer was similar in the

Table III Haemodynamic data at rest sitting for patients in later stages of myocardial infarction and for

Myocardial infarction	Age (yrs)	No. of pat.	Heart rate (beats/min)	Cardiac output (l/min)	Stroke vol. (ml)
Controls	51	14	63 ± 3	5.6 ± 0.2	91 ± 3
16th to 43rd day	60	16	71 ± 3	4.8 ± 0.2	0 ± 4
3rd to 13th month	53	19	70 ± 2	4.6 ± 0.1	67 ± 2
1st to 7th year still at work	51	16	67 ± 2	5.3 ± 0.2	80 ± 3
3rd to 11th year given up work	58	8	67 ± 3	4.6 ± 0.3	70 ± 5

Table IV Mean values of changes in haemodynamic data in patients with sinus rhythm investigated sitting

	Oxygen consumption (ml/min)	Heart rate (beats/min)	Cardiac output (l/mm)	Stroke vol. (ml)
No.	11	11	11	11
Mean of diff. ± SE of mean of diff.	+6 ± 8	−1 6 ± 3.6	−0.39 ± 0.33	−5.4 ± 3.3
Stand. dev	28	11.8	1.08	11 0
Probabil. of diff.	—	—	—	—

When comparison was strictly limited to the eleven patients investigated both at the end of hospital stay and at the end of convalescence brachial arterial pressures and resistance had increased but there was *no significant increase in arteriovenous* oxygen difference.

The haemodynamic development in patients *after infarction seems to be* characterised by more or less diminished cardiac function at rest in sitting position as it is reflected *in total blood flow measurements* at the end of convalescence some months after infarction compared with controls *This function is probably regained* a few years after infarction by patients who are able to return to work but not by patients who are *unable* to work. Blood pressure rises steadily probably due partly to small adjustments in the peripheral regulation of circulation i. e. peripheral resistance, and partly to *improved cardiac function* Exceptions to this rule are patients unable to return to work. The normal blood pressure together *with their significantly* higher peripheral resistance and lower cardiac output than in controls suggest that the *cause of the increased peripheral resistance* is probably the mentioned insufficient myocardial function How a *hidden hypertensive disease might* affect the increase in blood pressure and the development of myocardial insufficiency is *difficult to tell.*

Effect of change from recumbent to sitting position

Adjustment of circulation to a new body position may be influenced by

days and 5 to 15 weeks after myocardial infarction (Mean values and SE of means are given)

Oxygen consumption (ml/min)	Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
		Systolic	Diastolic	Mean			
+ 4 ± 4	+5 ± 2	+1 ± 3	+2 ± 2	+1 ± 2	+2 ± 1	−1.4 ± 0.8	− 9 ± 6
+11 ± 3	+9 ± 1	−5 ± 2	−3 ± 1	−4 ± 1	+2 ± 0	−1.1 ± 0.2	−15 ± 3
+ 6 ± 4	+8 ± 1	−2 ± 1	−2 ± 1	−4 ± 1	+2 ± 1	−1.1 ± 0.2	−19 ± 4

and 1 to 7 years after falling ill and for healthy men of similar age working on a bicycle ergometer with

Arterio-venous oxygen diff. (ml/l)	Brachial arterial pressure (mm Hg)			Resistance (units)	Left ventricular work (kpm/min)	Left ventricular stroke work (pm)
	Systolic	Diastolic	Mean			
95 ± 4	102 ± 4	89 ± 2	116 ± 2	13.0 ± 0.8	14.6 ± 0.6	158 ± 8
106 ± 2	137 ± 5	80 ± 3	109 ± 4	13.8 ± 0.6	11.9 ± 0.4	122 ± 6
110 ± 4	156 ± 6	84 ± 6	112 ± 6	14.0 ± 0.7	12.6 ± 1.1	122 ± 9

after than before infarction. The relatively longer period of reduced physical activity (1—7 years) may have contributed more decisively to poorer exercise tolerance than in patients after convalescence. Some inhomogeneity in the two series with respect to the severity of the disease may also have played a contributory role.

Summary

Haemodynamic data have been recorded at rest and during exercise at the end of convalescence from myocardial infarction, and compared with values for the same patients in the acute stage and before they left hospital with values for other patients with myocardial infarction investigated after they had returned to work, and with values for controls.

At the end of convalescence the cardiac output was similar to that in the acute stage and before the patients left hospital and lower than in controls and in patients investigated 1—7 years after infarction when they had resumed work. Stroke volume was higher than in the acut stage, similar to that before the patients left hospital and similar to that in the patients who had not resumed work. It was lower than in controls and in patients who had resumed work. Arteriovenous oxygen difference was higher than in controls and in patients at the end of the hospital stay.

The differences in circulation when patients were lying and sitting were similar to those in patients before they left hospi-

Table V Differences in haemodynamic data between recumbent and sitting in controls and patients 16 to 43

Myocardial infarction	Age (yrs)	No. of pat.	Heart rate (beats/min)	Cardiac *output* (l/min)	Stroke vol. (ml)
Controls	50	12	0 ± 2	-0.7 ± 0.3	-11 ± 4
16th to 43rd day	60	14	0 ± 1	-0.6 ± 0.1	-9 ± 2
3rd to 13th month	53	18	$+2 \pm 1$	-0.7 ± 0.1	-12 ± 3

Table VI. Comparison of haemodynamic data for male patients with a myocardial infarction 3 to 13 months a load of 200 kpm/min (Means and SE of means are given)

Myocardial Infarction	No.	Oxygen consumption (ml/min)	Heart rate (beats/min)	Cardiac *output* (l/min)	Stroke vol. (ml)
Controls	10	869 ± 31	94 ± 5	9.2 ± 0.4	99 ± 5
3rd to 13th month	16	843 ± 16	100 ± 4	8.0 ± 0.2	82 ± 4
1st to 7th year still at work	10	889 ± 44	106 ± 5	8.1 ± 0.3	78 ± 5

controls, the patients of the present study and the patients working 1—7 years after infarction Cardiac output in the patients of the present study was similar to that of the patients investigated after they had resumed work. Both groups of patients had lower output than the controls. The same applied to the absolute values of stroke volume during work. Arteriovenous oxygen difference during work was higher in the patients of the present study than in the controls and the same as in the patients working 1—7 years after infarction. The mean arterial blood pressure was similar in the three groups, and so was the resistance.

Cardiac output, higher at rest in patients working 1—7 years after infarction than in the patients of the present study and of the same order as in controls, did not increase during exercise as much as in the present patients. Furthermore, the comparatively low stroke volume at rest increased during exercise in the present patients while the comparatively higher stroke volume in patients working 1—7 years after infarction decreased somewhat. These differences in haemodynamic response to exercise between patients in different phases of infarction and controls suggest that the exercise tolerance is quite well regained at the end of convalescence but may become worse later A decreased myocardial muscular mass may more easily become insufficient when circulation is stressed by exercise. Another factor of importance could be that patients avoided physical exercise more

Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Diagnostic Use of Fractionated Lactic Dehydrogenase Activity (LDH) in Myocardial Infarction

By

C. Erik Moller and E. Raabo

The diagnosis of myocardial infarction is generally based on the history the physical examination of the patient, pulse rate, blood pressure temperature curve, ECG and number of laboratory tests.

In the literature there are variations in the reliability with which the in vivo diagnosis of infarction has been made in patients who have died of myocardial infarction The diagnosed cases range from 60 (9) to 93 % (5) The ECG tracings are stated to have shown an infarction pattern in only one-half to two-thirds of the cases (8, 13) It is reported that measurement of temperature, sedimentation rate, and white cell count are non-specific investigations which are, however of value if repeated determinations are made during the first days after the infarction (1 5)

The diagnostic accuracy has increased since the introduction of the serum glutamic-oxalacetic acid transaminase (SGOT) test. Recent research has shown however that this test is lacking in specificity if it is to be used in diagnosing myocardial infarction. Elevated values have been found, for instance, in myocarditis, in patients with cardiac decompensation without myocardial infarction (2) postoperatively (12) in acute hepatitis, cirrhosis, obstructive hepatitis, centrilobular liver cell necrosis, acute pancreatitis, metastatic neoplasms, pulmonary embolism haemolytic diseases, a few cases of dissecting aortic aneurysm (19) injuries and burns. One author has reported a number of elevated values of which he found no pathological explanation his series consisted of elderly patients (20)

Several authors (15 29 31) have found the lactic dehydrogenase activity (LDH) in the serum elevated in 100 % of patients with myocardial infarction It is added that the diagnosis must be looked upon with some scepticism if the LDH is not elevated (17) This test too is stated to be non-specific, elevated values being found also in burns, injuries, post operatively in hepatitis, cirrhosis, centrilobular liver cell necrosis, decompensated

Submitted for publication June 17 1963.

tal A small decrease in brachial arterial mean pressure seen in the patients was not encountered in young controls

Cardiac output and stroke volume during light exercise was lower than in controls and similar to that in patients investigated 1—7 years after infarction when they had resumed work. The change in stroke volume was similar to that in controls. The arteriovenous oxygen difference was higher in both groups of patients than in controls

Acknowledgment

This study was supported by a grant from the Swedish National Association against Heart and Lung Diseases.

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On the basis of the above-mentioned findings, a number of authors have studied the LDH isoenzyme distribution in the serum in diseases involving an increase in LDH. The increased activity is assumed to be due to a release of enzyme from cells, due either to cellular breakdown or to an alteration in the permeability of the cell membrane.

An abnormal distribution of activity with an increase in the fast moving fractions, has been found in myocardial infarction (35) untreated pernicious anaemia and haemolytic anaemia (6) and in muscular dystrophy (22) while a distribution pattern with a preponderance of the slow-moving fractions has been found in hepatic diseases (37) pancreatitis (7) and pulmonary embolism (32)

A distribution pattern characterized by an increase in the middle fractions has been found in leukaemia (11) and malignant neoplasms (7)

It was the object of the present study to investigate the diagnostic value of the LDH isoenzyme distribution in myocardial infarction.

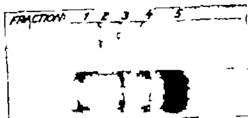


Fig 1. Distribution of LDH isoenzymes in human serum in relation to the serum protein distribution.

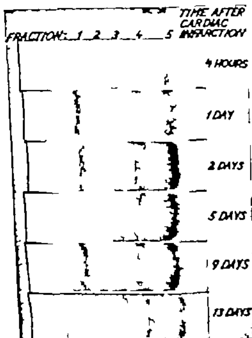


Fig 2. Distribution of LDH isoenzymes in the serum during the period following upon coronary thrombosis.

Methods

The method used for determining the LDH isoenzyme distribution in the serum is based on the use of the tetrazolium salt INT as redox indicator The principle of this method, which has been described elsewhere (26) will be mentioned very briefly

The isoenzymes are separated by paper electrophoresis at pH 7.5 and 10 olts/cm. Thereupon, the paper strips are incubated in reaction medium consisting of sodium lactate, diphosphopyridine nucleotide INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride) and phenazonium methosulphate. The stained enzyme-protein bands appearing on the paper are then determined photometrically On the basis of the total activity determined according to method described previously (25) and the per cent distribution, the absolute activity may be calculated for each fraction.

Normal values. For total LDH the normal range was fixed as 15–40 units/ml serum. The error in single determination has been found to be ± 5 On the basis of the LDH isoenzyme distribution in sera from 30 normal adult persons the normal values for the per cent distribution and absolute activity were calculated on the basis of the 99 % limit of

Table I Normal values for LDH isoenzyme activity and % distribution

	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
%	5—18	5—18	15—24	28—40	15—24
Activity units	1 0—5.6	2.2—5.6	3.7—9.9	5.8—13.2	4.2—7.4

heart disease, tuberculous pericarditis acute pancreatitis, stroke haemolytic conditions, untreated pernicious anaemia, lobar pneumonia systemic diseases, infectious mononucleosis, uraemia severe bronchopneumonia, pulmonary embolism and metastatic neoplasms (15 17 29 33 36) Thus the SGOT and LDH must be viewed with reserve in the event of a suspicion of myocardial infarction in patients who are decompensated are suffering from diseases of the liver or biliary tract or who have recently undergone operation Acute pancreatitis and lobar pneumonia are also impossible to differentiate definitely from myocardial infarction by means of SGOT and LDH Many writers have emphasized the importance of repeated determinations during the first days after the onset of the infarction (1 9 15 17 31) the SGOT rapidly increasing and reaching a maximum during the second day Normal values are seen again as early as the 5th—6th day (9) LDH shows an increase about 12 hours after the onset of the infarction the maximum being attained on the 3rd—6th day while normal values are not seen again until the 8th—14th day (29 31) In differentiating between cardiac and hepatic disease simultaneous determination of SGOT and serum glutamic pyruvic acid transaminase (SGPT) has been tried SGPT is said to show a relatively greater increase in the case of hepatic diseases. However the results of these investigations have been extremely varied (9 20)

Recent isoenzyme research forms the basis of a more specific method applicable as a diagnostic aid in myocardial infarction.

LDH isoenzymes

A number of papers published since 1950 have shown that several enzymes can be separated into a number of components having the same substrate specificity but differing in various physicochemical respects In 1952 Neilands (18) demonstrated that crystalline beef heart LDH could be separated into two fractions by electrophoresis, and subsequent authors have shown 4 or 5 LDH fractions having a different electrophoretic mobility in human serum (14 24 30 33, 35 37) In various human tissues it was possible also to demonstrate up to 5 fractions having LDH activity but the distribution varied from one tissue to another (10 21 22 23)

Markert and Moller in 1959 (16) suggested that these isodynamic enzymes of the same origin but having different physico-chemical properties be called isoenzymes or isozymes, and these designations have now been generally accepted

Recently (36) it has been demonstrated that one of the reasons for the difference in the electrophoretic mobility of the isoenzymes is a difference in amino acid composition among the isoenzymes of a given organ.

On the basis of the above-mentioned findings, a number of authors have studied the LDH isoenzyme distribution in the serum in diseases involving an increase in LDH. The increased activity is assumed to be due to a release of enzyme from cells, due either to cellular breakdown or to an alteration in the permeability of the cell membrane.

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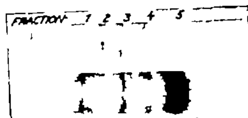


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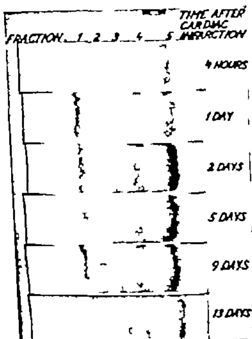


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A number of papers published since 1950 have shown that several enzymes can be separated into a number of components having the same substrate specificity but differing in various physico-chemical respects. In 1952 Neilands (18) demonstrated that crystalline beef heart LDH could be separated into two fractions by electrophoresis, and subsequent authors have shown 4 or 5 LDH fractions having a different electrophoretic mobility in human serum (14 24 30, 33 35 37) In various human tissues it was possible also to demonstrate up to 5 fractions having LDH activity but the distribution varied from one tissue to another (10 21 22 23)

Markert and Møller in 1959 (16) suggested that these isodynamic enzymes of the same origin but having different physico-chemical properties be called isoenzymes or isozymes, and these designations have now been generally accepted.

Recently (36) it has been demonstrated that one of the reasons for the difference in the electrophoretic mobility of the isoenzymes is a difference in amino acid composition among the isoenzymes of a given organ.

Table III. Distribution of the most important clinical diagnoses on the groups

Clinical diagnosis	Sex	Group I	Group II	Group III	Group IV
Coronary occlusion	♂	25	—	—	—
	♀	8	—	—	—
? Coronary occlusion	♂	—	6	—	—
	♀	—	5	—	—
Arterioscl. heart disease (no occlusion)	♂	—	—	17	—
	♀	—	—	12	—
Arterioscl. heart disease + dermatomyositis	♂	—	—	1	—
Arterioscl. heart disease + lobar pneumonia	♂	—	—	1	—
Arterioscl. heart disease + acute pancreatitis	♀	—	—	1	—
Arterioscl. heart disease + lymphosarcoma	♀	—	—	1	—
Systemic lupus erythematosus	♂	—	—	2	—
Cor pulmonale	♂	—	—	2	—
Mitral disease	♂	—	—	2	—
Hypertensive heart disease	♀	—	—	1	—
Acute pericarditis	♂	—	—	1	—
Cardiac neurosis	♂	—	—	—	4
	♀	—	—	—	3
True angina	♀	—	—	—	1
Lipothymia	♀	—	—	—	1
Cholelithiasis	♂	—	—	—	1
Cholecystitis	♂	—	—	—	1
Bronchial asthma	♂	—	—	—	2
Lobar pneumonia	♂	—	—	—	1
Fracture of ribs	♂	—	—	—	1
Humero-scapular periarthritis	♀	—	—	—	1
Embolism in pulm. artery	♀	—	—	—	1
Total		33	11	41	17

Autopsy was carried out on 16 of the 18 patients who died. The clinical diagnosis was confirmed in 14 while in two the diagnosis is based exclusively upon the post-mortem report.

An 86-year-old woman admitted with pulmonary oedema. ECG showed bundle-branch block. The temperature was not elevated. LDH 48 units. SGOT 26 24 units. The patient died within the first 24 hours after admission. Autopsy revealed fresh, blackish-red thrombus, 1 cm in length, in the descending branch of the left coronary artery. Moreover there was not entirely recent anterior-wall infarction with parietal thrombus. On section, the myocardium showed yellowish-white areas. Diagnosis: Recent and older myocardial infarctions.

An 82-year-old man was admitted in a state of severe decompensation. ECG revealed bundle branch block. The patient had an aortic stenosis and died 3 days after admission with pulmonary oedema. Severe hepatic congestion. SGOT 45 86 units. LDH 64, 73 units. Post-mortem examination showed aortic stenosis with multiple, recent myocardial infarctions. No vascular thrombosis.

The other patients of Group I had typical symptoms and definit ECG pattern of infarction.

Group II. Eleven patients in whom a diagnosis of myocardial infarction could not be made with certainty although the clinical signs were compatible with such diagnosis.

Table II Total series by age and sex Deaths in brackets

Group	Sex	Age						
		30—39	40—49	50—59	60—69	70—79	80—89	Total
I	♂	0	4 (1)	3 (1)	6 (3)	11 (8)	1 (1)	25 (14)
	♀	0	0	3 (2)	1	3 (1)	1 (1)	8 (4)
II	♂	0	0	1	1	4 (1)	0	6 (1)
	♀	0	0	0	2	2 (1)	1 (1)	5 (2)
III	♂	1	1	4	9	7 (1)	4 (1)	26 (2)
	♀	0	0	1	6	4 (1)	4 (2)	15 (3)
IV	♂	0	2	3	5	0	0	10
	♀	0	1	3	1	2 (1)	0	7 (1)
Total								102 (27)

probability (table I) The error in a single determination was found to be ± 10 %

SGOT was determined by the method of Reitman and Frankel (27) The normal range is 8—40 units/ml serum.

LDH isoenzyme distribution in normal subjects and in acute coronary infarction

Fig 1 shows the result of an isoenzyme fractionation of a serum having a normal activity Five separate fractions were found in all the sera studied.

Fig 2 shows the isoenzyme distribution in the serum during the period after acute myocardial infarction. This distribution pattern shows an increase in the fast moving fractions, especially fractions 4 and 5 It is evident from the figure that in this patient the distribution had not returned to normal in two weeks.

Material

The material consists of patients who were admitted to the Department of Medicine during the period June 15th to Oct. 15th, 1962, in a condition which suggested myocardial infarction.

The planned obligatory investigations for this series were as follows A detailed history repeated physical examinations, control of temperature, pulse rate, and blood pressure ECG with 6 precordial leads, ESR and white cell counts every day during the first 3 days, thereafter once weekly SGOT and total LDH daily during the first 3 days after admission, and fractionated LDH at least once, preferably on the day of the highest total LDH value. As far as possible, chest radiography was done at least once during the stay in hospital.

Autopsies were carried out in the Pathology Department by Dr J Ringsted

The clinicians were not acquainted with the results of the LDH fractionations until after the patients had been discharged. Thus, the diagnoses were based on all the usual clinical and laboratory studies, including autopsy — with the exception of fractionated LDH.

The classification of the series of 67 males and 35 females into 4 groups was based on these diagnoses. Table II gives the age distribution and sex ratio. The average age was 65 5 years for the males and 68 years for the females.

Group I comprises 33 patients with unmistakable myocardial infarction. As might be expected, the group has a male preponderance. The high mortality is explained by the fact that all patients were included, including 3 males (51 76, and 71 years of age) and one female (aged 56) who died immediately after admission before any blood samples had been obtained, so that these patients have to be left out of the further analysis of the material. In all four post mortem examination revealed myocardial infarction

Table IV Mean values for total LDH and fractionated LDH Minimum and maximum values in brackets

Group	Total LDH	Fraction				
		1	2	3	4	5
I	119 (51–446)	12.0 (1.7–53.6)	7.5 (0–26.9)	12.2 (5.1–35.8)	35.5 (16.0–116.5)	48.4 (11.5–215.0)
II	64 (33–102)	8.8 (2.3–26.0)	4.9 (0.9–9.8)	10.8 (2.5–22.3)	21.5 (10.4–37.7)	19.1 (6.4–33.8)
III	47.5 (26–118)	8.7 (2.1–42.2)	3.6 (0–13.1)	8.7 (2.6–22.6)	14.8 (5.8–30.0)	8.5 (2.9–17.4)
IV	48.1 (22–184)	14.3 (2.0–120)	3.7 (1.8–22.1)	8.8 (5.5–19.0)	13.3 (7.1–29.6)	6.5 (1.6–12.9)

In fractions 4 and 5 on fig 4 all the values are above the normal range. This makes a characteristic pattern, clearly distinguishable from the pattern found on figs. 6 and 7 The mean value for LDH in fractions 4 and 5 on fig 4 is the same, if only the fatal cases ar considered. From this it seems justified to conclude that the *increase in th activity of fractions 4 and 5 can hardly be of prognostic significance.* Fig 5 Group II) shows a pattern which is intermediate between that illustrated in figs 4 and 6, few values being within the normal range in fractions 4 and 5

Most of the values shown on fig. 7 (Group IV) are within the normal range.

Criteria for assessing fractionated LDH

T ble V shows all values of total LDH and *fractionated LDH* for the patients of G oup I

The typical infarction pattern, with an increase of fraction 4 and especially fraction 5 can usually be discerned with some practice from the strips. In order to be able to evaluate the results in questionble cases, where the total LDH is only slightly elevated, and in order to be ble to operate with a simple laboratory re port, we shall try on the basis of the values in table V to set up the following criteria for assessing fractionated LDH in myocardial Infarction

\+ indicates that at least 50 % of total LDH is made *up by the sum of fractions 4* and 5 and that fraction 5 is equal to or greater than fraction 4 in *terms of LDH* units.

(+) indicates that at least 50 % of the total LDH is made up by the sum of the elevated fractions 4 and 5 that the total LDH is above 40 units and *below* 60 units, and that fraction 5 is below fraction 4 in *terms of LDH units.*

— indicates all other patterns also cases in which the total LDH is above 60 units and fraction 5 below fraction 4

In view of the small size of the series the named criteria were not set up on the basis of an ordinary statistical evaluation.

The criteria applied to Group II

The 11 patients of this group will be briefly described.

1 A 69-year-old woman on digitalis because of decompensated *arteriosclerotic* heart disease. For the past 10 years the patient had suffered from attacks of pain below the left costal border precipitated by physical exertion and cold. She had never used ultro-

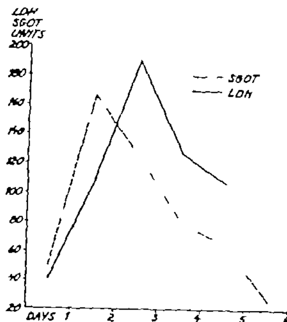


Fig 3 Mean values for total LDH and *SGOT in* the Group I patients during the first 5 days after the onset of infarction.

ECG did not in any case show an infarction pattern.

Three died, but only one was subjected to autopsy

A 74-year-old woman was admitted with pulmonary oedema. ECG revealed atrial fibrillation. The patient died 24 hours after admission. SGOT 31 125 units. LDH 36, 58 units. Autopsy revealed coronary thrombosis, but no definite signs of infarction, even upon microscopic examination of the myocardium. The liver was yellowish pale and congested.

Group III Fourty-one patients with organic heart disease without definite clinical or laboratory signs of myocardial infarction Twenty-three had decompensated heart diseases, including 13 in whom the liver was palpable. By mistake the LDH fractionation was not carried out in the case of one patient, a 72 year-old woman, who must therefore be left out of the further analysis of the material.

A total of 5 patients of this group died. One was autopsied

An 80-year-old man with chronic, decompensated arteriosclerotic heart disease The patient died in a state of pulmonary oedema 12 days after admission. Autopsy revealed extensive coronary sclerosis, but no stenosis or thrombus. Gross and microscopic inspection of the myocardium showed no definite signs of myocardial infarction.

Group IV comprises the remainder of the material, a total of 17 patients. On admission these patients might be presumed to have myocardial infarction but the investigations disclosed that the symptoms and signs were caused by extracardial diseases. One death occurred

A 79-year-old woman was admitted two days after she developed chest pain, fever dyspnoea, and cyanosis. ECG showed a pattern like that seen in posterior wall infarction. SGOT 150, 1 040 units. LDH 62 184 units. White cell count 19 600 16,200, 17 100. The patient died 2 days after admission. The clinicians believed that the death was caused by myocardial infarction but autopsy revealed emboli in the pulmonary arteries (issuing from deep thrombophlebitis of the right leg) Microscopic examination revealed liver cell necrosis. No myocardial infarction was demonstrable.

The most important diagnoses in all 4 groups are given in table III In addition to the diagnoses listed, Groups I—II included one and Group III three patients with diabetes mellitus.

In our material too the total LDH was elevated in 100 % of the patients with definite infarction. But the fact that the test is non-specific may be seen int. al. from the finding that three-quarters of the patients of Group III had elevated total LDH In Group I as well as Group III 70 had an ESR higher than 10 mm on admission. The values for total LDH and SGOT in the Group I patients during the first days after the onset of infarction are shown in fig 3.

Results

Table IV shows the mean values of total and fractionated LDH in the four groups The individual results are presented graphically in figs 4—7 A comparison of fig 4 (Group I) with fig 6 (Group III) shows a decisive difference

Table IV Mean values for total LDH and fractionated LDH Minimum and maximum values in brackets

Group	Total LDH	Fraction				
		1	2	3	4	5
I	119 (51–446)	12.0 (1 7–53 6)	7.5 (0–26.5)	12.2 (3.1–33.6)	33.3 (16.0–116.5)	48.4 (11.3–215.0)
II	64 (33–102)	8.8 (2.3–26.0)	4.9 (0.9–9.8)	10.8 (2.5–22.3)	21.5 (10.4–37 7)	19.1 (6.4–33.8)
III	47.5 (20–119)	8.7 (2.1–42.2)	5 6 (0–13.1)	8.7 (2.6–22.6)	14.8 (5.8–38.0)	8.5 (2.9–17 4)
IV	48 1 (22–104)	14.3 (2.0–123)	5.7 (1.8–22.1)	8.8 (5.5–18.0)	13.3 (7 1–29.6)	6.5 (1.6–12.9)

In fractions 4 and 5 on fig 4 all the values are above the normal range. This makes a characteristic pattern, clearly distinguishable from the pattern found on figs. 6 and 7 The mean value for LDH in fractions 4 and 5 on fig. 4 is the same, if only the fatal cases are considered. From this it seems justified to conclud that the increase in the activity of fractions 4 and 5 can hardly be of prognostic significance. Fig 5 (Group II) shows a pattern which is intermediate between that illustrated in figs. 4 and 6 few values being within the normal range in fractions 4 and 5

Most of the values shown on fig 7 (Group IV) are within the normal range.

Criteria for assessing fractionated LDH

Table V shows all values of total LDH and fractionated LDH for the patients of Group I

The typical infarction pattern with an increase of fraction 4 and especially fraction 5 can usually be discerned, with some practice, from the strips In order to be ble t evaluate the results in questionble cases, where the total LDH is only slightly elevated, and in order to be able to operate with a simple laboratory report, w shall try on the basis of the values in table V to set up the following criteria for assessing fractionated LDH in myocardial infarction

+ indicates that at least 50 % of total LDH is made up by the sum of fractions 4 and 5 and that fraction 5 is equal to or greater than fraction 4 in terms of LDH units.

(+) indicates that at least 50 / of the total LDH is made up by the sum of the elevated fractions 4 and 5, that the total LDH is above 40 units and below 60 units, and that fraction 5 is below fraction 4 in terms of LDH units.

— indicates all other patterns, also cases in which the total LDH is above 60 units and fraction 5 below fraction 4

In view of the small size of the series the named criteria were not set up on th basis of an ordinary statistical evaluation.

The criteria applied to Group II

The 11 patients of this group will be briefly described.

1 A 69-year-old woman on digitalis because of decompensated arteriosclerotic heart disease. For the past 10 years the patient had suffered from attacks of pain below the left costal border precipitated by physical exertion and cold. She had never used nitro-

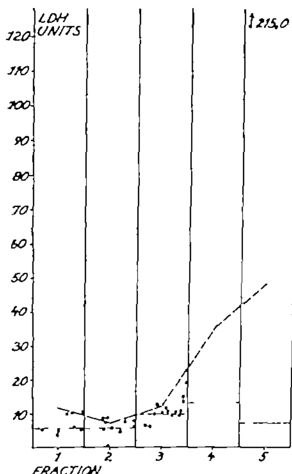


Fig 4 Results of fractionated LDH in the Group I patients (a total of 29) The horizontal broken lines indicate the upper limit of normal values. The roughly broken line connects the mean values.

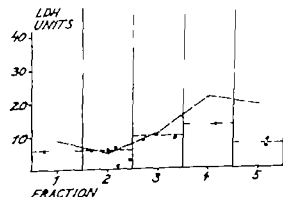


Fig 5 Results of fractionated LDH in the Group II patients (a total of 11) (For symbols, cf. fig 4)

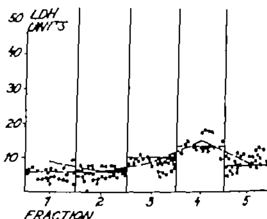


Fig 6. Results of fractionated LDH in the Group III patients (a total of 40) (For symbols, cf. fig 4)

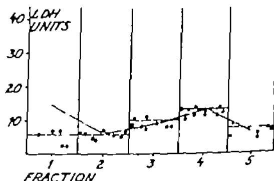


Fig 7 Results of fractionated LDH in Group IV patients (a total of 17) (For symbols, cf. fig 4)

glycerin tablets. She was admitted after 5 days of pain requiring morphine injections. During the first two weeks she was running a low-grade temperature. There was thrombophlebitis of the lower limbs. ECG showed bundle-branch block. No objective signs of decompensated heart disease. Intravenous pyelography gave rise to a suspicion of a small calculus in the left ureter Pyuria, isosthenuria. SGOT 22, 63, 56 units. LDH 67 77 102 units. The total LDH of 102 units was fractionated (fractions 1–5) 14.3, 4 1 8.2, 37 7 37 7 — +

2 A 69-year-old man, who had been receiving disablement pension for 6 years because of arterial hypertension During recent years occasionally retrosternal pain on exertion The patient was admitted after having

Table V All values for total LDH and fractionated LDH in the Group I cases. + (+) — results in the column on the right

Total LDH	Fraction					
	1	2	3	4	5	
150	28.5	10.5	15.0	30.0	57.0	+
53	5.3	4.8	9.5	18.0	15.4	(+)
59	5.5	7.4	12.4	21.3	13.0	(+)
54	5.9	4.9	7.8	18.9	17.3	+
51	5.1	7.7	10.7	16.3	11.3	(+)
254	22.9	13.2	27.9	81.2	108.8	+
458	53.8	26.9	35.8	116.5	215.0	+
204	10.2	6.1	12.2	90.0	86.0	+
86	3.4	0.0	6.9	37.9	37.9	+
212	31.8	19.1	31.8	61.5	67.8	+
123	3.7	8.6	13.5	44.2	53.0	+
108	3.2	4.3	6.5	44.3	49.8	+
74	5.2	3.7	10.3	24.4	30.2	+
81	3.2	2.4	8.1	29.2	38.1	+
86	10.3	7.7	12.9	22.4	32.7	+
104	10.4	5.2	10.4	33.3	44.8	+
117	10.8	11.7	14.0	32.8	47.9	+
76	3.0	2.5	6.1	26.6	38.0	+
212	12.7	4.2	19.1	80.6	95.5	+
100	10.0	10.0	10.0	28.0	42.0	+
88	3.6	1.8	8.9	32.0	42.0	+
145	18.8	8.7	5.8	55.0	56.5	+
83	1.7	1.7	5.1	32.3	44.2	+
121	23.4	12.1	17.0	32.7	34.0	+
111	17.8	10.0	13.4	26.6	43.2	+
68	8.8	6.8	10.2	19.7	22.4	+
57	9.1	5.7	9.7	16.0	16.5	+
67	10.1	4.6	7.4	21.4	25.1	+
67	10.7	4.0	6.7	21.5	24.1	+

had an attack of retrosternal oppression with dyspnoea for 3 days. On admission the blood pressure was 230/170, but at the end of few days 180/100. ECG abnormal, but no infarction pattern. Temperature normal. ESR 7 9 13 mm/h. SGOT 60, 20, 38, 29 units. The pain had disappeared on the day after admission, and the patient was feeling well when discharged three weeks later. LDH 63, 44 57 55 units. The total LDH of 55 units was fractionated (fract. 1—5) 5.8, 5.8, 8.1 17.4 20.9 — —

3. A 74-year-old man with diabetic nephropathy. Admitted after sudden attack of dyspnoea associated with retrosternal pain. On admission the patient had pulmonary oedema, hepatomegaly and peripheral oedema. Favourable effect of digitalis. Low grade fever during the first 5 days. ECG abnormal, but no infarction pattern. ESR 12, 15, 14 mm/h. LDH 56, 48, 58, 52 units. SGOT 10 48, 42, 22 units. The total LDH of 58 units was fractionated (1—5) 5.8, 5.8, 8.1 17.4 20.9 — +

4 An 80-year-old female. During the past 7 years she had been admitted a total of 10 times for arteriosclerotic heart disease with bundle-branch block and atrial fibrillation. This time, she was admitted after having had increasing dyspnoea and precordial oppres-

sion for 3 days. Pulmonary oedema on admission. On the day of admission she developed an embolus in the left popliteal artery and 3 days later cerebral embolism. She died one week after admission. Autopsy was not performed. SGOT 31 118 54 units. LDH 55 90 units. The total LDH of 90 units was fractionated (1—5) 2 7 0 9 14 4 33 3 38.8 = +

5 A 55-year-old man with cardiac neurotic complaints for the past 20 years. Admitted after a nocturnal attack of retrosternal pain no dyspnoea or cyanosis. During his stay in hospital the patient was afebrile and his general condition unaffected On the second day of the illness ECG showed transient inversion of T_1 SGOT 22 80 61 22 units. ESR Below 10 on 8 occasions. LDH 39 42, 51 units. The total LDH of 51 units was fractionated (1—5) 4 6 2.5 2.5 18 9 22 5 = +

6 A 73-year-old man on digitalis for decompensated arteriosclerotic heart disease with bundle branch block of about one year's duration. Four months prior to the present illness the patient had been admitted with pulmonary oedema. Now he was re-admitted in a state of severe decompensation. ECG showed bundle-branch block. The patient suddenly died 17 days after admission. No autopsy SGOT 20 units. LDH 41 38 units. The total LDH of 38 units was fractionated (1—5) 2 3 3 0 8 4 15.2 9 1 = (+)

7 A 72 year-old man who had been suffering from pressure pain in the chest on exertion for the past 3 months. Two hours prior to admission sudden retrosternal pressure associated with dyspnoea. During the stay in hospital no particular symptoms. Died 3 months after discharge of coronary occlusion confirmed post-mortem ECG showed negative T_1 which remained unchanged on repeated investigations. SGOT 19 125 75 units. LDH 40, 82 78 units. Regrettably only the LDH of the first day was fractionated (1—5) 10.4 5 6 7.2 10.4 6 4 = —

8. A 63-year-old woman admitted because of sudden retrosternal pain associated with dyspnoea. General condition unaffected during the stay in hospital. ECG normal. No fever SGOT 43 100 380 units. LDH 37 33, 36 units. Eight days after admission SGOT was normal. Other tests gave no explanation of the pain or the transient elevation of the SGOT The total LDH of 33 units was fractionated (1—5) 3 6, 2.6, 5 6, 13.9, 7.3 = —

9 A 74-year-old man with chronic pyelonephritis and azotaemia. Also aortic stenosis of unknown genesis. Admitted in a state of pulmonary oedema. Favourable effect of digitalis. SGOT 10, 50, 22, 16 units. LDH 32, 68 93 54 units. The total LDH of 93 units was fractionated (1—5) 5.6, 9.3, 22.3, 36.3, 19.5 = —

10 A 72 year-old woman who had been suffering from attacks of retrosternal pain on exertion for the past 8 years. Usually a good effect of nitroglycerin. Admitted after ? days constant pain not responding to nitroglycerin Not debilitated on admission. No dyspnoea or cyanosis. During 2 months in hospital she had repeated attacks of pain, sometimes responding to nitroglycerin. ECG abnormal, but unchanged and without an infarction pattern. SGOT and LDH tested repeatedly with results ranging from normal to slightly elevated values. The highest total LDH of 78 units was fractionated (1—5) 14 8, 6.3 11 7 23 4 21.8 = +

11 This was the above-mentioned 74-year-old woman who died 24 hours after admission Autopsy revealed coronary thrombosis, but no definite infarction, even on microscopic examination of the myocardium. The total LDH of 70 units was fractionated (1—5) 26 0 9.8, 12.6, 13.3 8.4 = —

Six patients showed the same isoenzyme pattern as the Group I patients. We believe that these 6 patients had myocardial infarction.

Four patients had isoenzyme patterns different in distribution One had a pattern which we have designated as (+)

In case 7 only the first-day LDH was fractionated The result might have been different if the elevated total LDH from the 2nd or 3rd day had been fractionated.

Case 11 is remarkable in having had coronary thrombosis without myocardial infarction.

The criteria applied to Group III

No case of this group received the designation + on fractionated LDH determination. Twenty-nine were — and 11 (4 males and 7 females) were (+)

The four males were 69 71 78, and 80 years of age. The last mentioned patient died in pulmonary oedema and has been described above. Autopsy revealed coronary sclerosis but no myocardial infarction. The patient aged 78 years had previously been admitted with myocardial infarction and pulmonary oedema. This time he was admitted in a state of decompensation after a 3-day attack of precordial pain. Definite clinical or laboratory signs of myocardial infarction could not be demonstrated. The patient died suddenly in his home 2 months after his discharge. The remaining two men had pulmonary oedema at the time of admission, and one had bundle-branch block.

The 7 women ranged in ge from 56 to 86, and all were suffering from arteriosclerotic heart disease Six were de compensated on admission.

The designation (+) we take to mean suspicion of myocardial infarction

The criteria applied to Group IV

All 17 patients of Group IV received the designation — upon LDH fractionation

One had total LDH of 184 Fractionation showed a marked elevation of fraction 1 (123 units) The patient was described above. The clinicians believed that he was suffering from myocardial infarction, but autopsy revealed an embolus in the pulmonary artery and centrilobular liver cell necrosis. As already mentioned, fractionated LDH was — i. e. in keeping with the post-mortem diagnosis.

In our opinion, determination of fractionated LDH is so far the most specific investigation for diagnosing myocardial infarction in questionable cases. A similar isoenzyme pattern may be found in untreated pernicious anaemia and in haemolytic disorders, but these conditions need rarely be considered in the differentiation from myocardial infarction

Summary

It is pointed out that the most common methods for diagnosing myocardial infarction are rather non-specific.

A practical, clinically applicable method for determining the LDH isoenzyme fractions following electrophoretic separation has been worked out. Fractionated LDH was determined on 97 patients admitted for evaluation with symptoms of myocardial infarction. The clinicians were not acquainted with the result of the LDH fractionation until they had diagnosed the individual cases on the basis of the usual investigations — including autopsy The material was divided into four groups I Patients with definite myocardial infarction. II Questionable cases. III Patients with organic heart disease without infarction. IV The remaining cases.

It is demonstrated that the patients with myocardial infarction had a special LDH isoenzyme pattern, consisting mainly in an increase of fractions 4 and 5 An ttempt was made to differentiate this pattern from all others by certain criteria according to which the results are given as + (+) or —

In Groups III and IV there were no + results. In Group II a + result was found in 6 out of 11 patients

One patient of Group IV who had an elevated total LDH and SGOT as well as an ECG pattern of infarction had been assumed by the clinicians to be suffering from coronary thrombosis. Autopsy revealed an embolus in the pulmonary artery Fractionated LDH had given the result —

We feel that fractionated LDH is the most specific laboratory test so far available for diagnosing myocardial infarction in questionable cases.

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Bronchial Adenomas and the Carcinoid Syndrome

By

Alf Ahlgren and Lars Hillerdal

Primary carcinoid tumours are generally seen in the gut and especially in the ileocoecal region. They are considered to derive from the enterochromaffine cell system, the cells of which are responsible for the normal synthesis of serotonin. The connection between the serotonin-producing tumour and the flush attacks, gastrointestinal symptoms and right-sided heart changes was established in 1954 by Thorson et al. (31) and in 1955 by Waldenström and Ljungberg (33) and was called the carcinoid syndrome. Since then the biochemistry, physiology and clinical symptoms of serotonin have been dealt with by several authors (7, 22, 28, 29, 32).

The first cases of a bronchial carcinoid with a carcinoid syndrome were published in 1958 (6, 14, 26, 30, 3) but already in 1956 Kincaid-Smith and Brossy (15) related a case of bronchial carcinoid with liver metastases, such that a carcinoid syndrome must be suspected. Until 1960 the opinion was generally held that liver metastases are essential to constitute the carcinoid syndrome. In that year, however, Joseph and Taylor (12) reported a case of bronchial carcinoid where autopsy excluded liver metastases but which nevertheless showed the actual symptom complex. Some other cases without liver metastases have later been published (8, 26).

The carcinoid type constitutes about 83 % of the bronchial adenomas. Like the carcinoids of the small intestine only a few are endocrinely active (functioning) as evidence by a more or less acute hypersecretion of serotonin. The carcinoid syndrome is caused by this hyperserotoninaemia and is characterized by symptoms from different organs, especially the skin, the bronchi, the digestive tract and the heart.

Case report

70-year-old man, formerly factory worker. 1944 a gangrenous appendicitis, no carcinoid tumour at histological examination. 1947 peptic ulcer. Since 1920 frequent bronchopneumonias on the right side but not until 1953 was he examined bronchoscopically. There was then found a bronchial carcinoid in the apical bronchus of the right lower lobe.

Submitted for publication June 18, 1963.

In Groups III and IV there were no + results. In Group II a + result was found in 6 out of 11 patients.

One patient of Group IV who had an elevated total LDH and SGOT as well as an ECG pattern of infarction had been assumed by the clinicians to be suffering from coronary thrombosis. Autopsy revealed an embolus in the pulmonary artery. Fractionated LDH had given the result —

We feel that fractionated LDH is the most specific laboratory test so far available for diagnosing myocardial infarction in questionable cases.

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Table I. Hitherto published cases of bronchial adenoma with a carcinoid syndrome with reference to year of publication, age, sex, symptoms and amounts of 5 HIAA in urine

Author	Sex	Age	Symptoms				5 HIAA in urine mg/24 h
			Flush	Asthma	Diarrhoea	Endocardial fibrosis	
Kincaid Smith & Brown (1959) Williams & Azzopardi (1960)	♀	58	+	−	+	?	74−100
Dockerty et al. (1958) Sauer et al., case 3 (1958)	♀	54	+		+	?	"pos.
Kahler et al. (1958)	♀	49	+	+	+	?	201
Sauer et al. (1958)	♀	53	−	+?	−	?	?
Do.	♀	66	−	+?	−	?	?
Bomford et al. (1958)	♀	42	+		+	?	38−46
Warner & Southren (1958)	♀	48	+	+	+	−	5−187
Do. (1958)	♀	55	+	+	+	?	"pos.
Bieler (1959)	♂	52	+		+	+	78−127
Lechnit & Wenzl (1959)	♀	31	+	+	−	+	arised"
Schmeckloth et al. (1959)	♂	45	+		+	−?	51−107
Anlyan et al. (1960)	♂	39	+	−	−	+	6.8 mg/100 ml
Grambach & Wiethoff (1960)	[illegible]	52	+	−	+	−	78−127
Joseph & Taylor (1960)	♀	62	+	+	+	−	28−40
du Mesnil de Rochemont & Lauth (1960)	♂	46	+	+		?	neg
Do. (1960) Gerck & Müller (1960)	♂	57	−	−	+	+	40−66
Reingold & Escovitz (1960) Escovitz & Reingold (1961)	♂	35	+	−	−	−	27
Hämelmann & Wendt (1961)	♂	59	+	−	+	−	40−80
Luparello & Mc Allister (1961)	♀	50	+	+	+	−	137
Noble (1961)	♂	47	+	+	+	+	320−600
Sandler et al. (1961)	♀	52	−	−	+	−	81
Weaver et al. (1961)		−	+	+	+	−?	
Went & Ingram (1961)	♂	32	+		+	+	?
Mc Conaghie (1962)	♀	63	+	−	+	+	17
Nasser Hampe et al. (1962)	♂	70	+	−	+	−	"Increased"
Pollard et al. (1962)	♀	56	+	−	−	−	139
Adlergren & Hillerson (1963)	♂	70	+	+	+	−?	86−470

During these [illegible]ttacks the hemoglobin decreased (1[illegible] 2 g [illegible]) as well as the hematocrit ([illegible] from 3 %) and [illegible]t the same time the diuresis was considerably diminished and the patient gained in weight 2−3 kg. The attacks came without any evident cause, but could be provoked by means of e. g. beer with an alcohol content of 3.5 [illegible] olume [illegible]. The amount of excreted 5HIAA was during the attacks 86, 88, 435 and 470 mg/24 h while in [illegible] symptom-free state 90−143 mg/24 h.

Radiation therapy was tried and the patient got 2,500 [illegible] over the site of the tumour. As expected no roentgenological or curative effect was seen and the patient's attacks continued unchanged. Nor did bronchoscopic examination reveal any improvement and the tumour growth was found to have increased considerably in both main bronchi.

Cyproheptadine (periactin) in a dose of 4 mg 2 times daily had [illegible] good effect on the diarrhoea but not on the other symptoms.

Excision of the right lower lobe in the same year microscopical examination revealing no signs of tumour growth at the resection surface or in neighbouring lymph nodes. Some suspected tumour cells were however seen in a lymph vessel not far from the resection border Uneventful postoperative course and no symptoms until 1957 when he began to cough and sometimes had blood tinged sputum. X ray of the lungs revealed then a right-sided dorsally localized infiltration with atelectasis At a new bronchoscopy with biopsy a tumour relapse was seen in the main and stem bronchi of the right side whilst the left side was inspected without remarks At this time the patient's lung function was not satisfactory but nevertheless he refused further operation.

The patient's state thereafter almost unchanged until the beginning of 1961 when he begun complaining of attacks of heat sensations in his face combined with a bright red colour The duration of these attacks was about 2 hours, but the duration frequency and intensity progressively increased and the redness tended to extend over still larger parts of the body Diarrhoea also developed during the attacks. At his admittance to the medical thoracic clinic in June 1961 the intervals between the attacks were 8—9 days and their duration 2 days.

Present status somewhat thin man without signs of incompensation. Curved nails. Lymph-nodes in front of the sternocleidomastoideal muscle and in the axilla bilaterally several lymph-nodes up to the size of an almond. The heart was displaced to the right but showed nothing notable even on auscultation. Blood pressure 130/85 Heart frequency 70 per minute. Over the right lung generally diminished breathing sounds with an obvious bronchial tendency No rales or rhonchi Dullness was percussed dorsally and laterally The left lung unremarkable. The liver could probably be palpated 3 cm below the right costal margin. Otherwise normal conditions in the abdomen The prostate gland somewhat enlarged but without any evidence of malignancy Neurologically no remarks. Normal temperature. X ray revealed a right lung which was totally atelectatic and there was a replacement of the mediastinum 3—4 cm to the right. Left lung without remarks. Bronchoscopy the lowest 2—3 cm of the front wall of the trachea were seen infiltrated by a fine granulated, grey red, easy bleeding tumour continuing down in the left main bronchus, the orifice of which was considerably diminished. In the right main bronchus extensive tumour growth was seen up to the carina. Parts of the tumour were removed and the passage in the left main bronchus was considerably improved. Biopsy and histological examination verified the diagnosis bronchial carcinoid. Complete radiological examination of the digestion tract was without remarks. Liver puncture and laparoscopy did not reveal any signs of liver metastases but the left part of the liver could not be inspected because of a colon loop Visible parts of the intestine were without remarks.

Laboratory investigations hemoglobin 9.2—12.5 g% Hematocrit 26—37% WBC 2,500—10,800 with a normal distribution. Blood platelets 158 000—304 000 ESR 51—100 mm/1 h. Serum protein 7.3 g Serum electrophoresis with somewhat low content of albumin and an increased gammaglobulin fraction (46 4 and 27 4 respectively in relative %) Blood electrolytes within normal limits. Urine sometimes traces of protein and 5—25 red cells per high-power field. Blood sugar ranging from 96 to 125 mg % during 24 hours. Meulengracht's test 1 4 Thymol turbidity test 2 units. Alkaline and acid phosphatase 6 and 1 unit respectively Hijman v d. Bergh's test negative. SGOT 8, SGPT 10 and SLD 36 units. Prothrombin-proconvertin 94 of normal (Owren) Serum cholesterol 238 mg % ASL 40 units, ASTA somewhat elevated to 8 units. Normal excretion of 17 ketosteroids (2.3—3.2 mg/24 h) and catecholamines (noradrenaline and adrenaline 29 and 3 μg/24 h respectively) in urine 3-methoxy-4-hydroxy mandelic acid in urine elevated 2—3 times (20—26.3 mg/l) Tryptamine excretion was 116—153 μg/l urine (normal value)

ECG at rest without notable features. Total blood volume during the attacks 6.6 l and when having no symptoms 6.2 l against an estimated normal value of 4 0 l

During his stay in the hospital the patient had repeated attacks with bright red discol ouration over the whole body increased tear flow obstruction in the nose diarrhoea and meteorism, ankle oedema and asthmatic symptoms such that rales and rhonchi could be auscultated.

right-sided one. This corresponds better with expectation, and it can be assumed that the primary tumour and its extra portal metastases produce serotonin in amounts such that not all of it can be eliminated in the liver or the lungs.

In the first instance operation is recommended when possible. In cases where this is not possible or where there is a relapse after operation virtually nothing remains at present but symptomatic therapy With variable results ergotamine tartrate, iproniacid, antihistaminic drugs, chlorpromazine, derivatives of rauwolfia serpentina and lysergic acid have been tried all of them interfering with the metabolism of serotonin in different ways. Heparin is said to have a depressive effect on serotonin metabolism and has been tried with at least a temporarily good result (2) Alpha-methyldopa prevents the transformation of 5-hydroxy tryptophan to serotonin and has been effective to a certain degree in some few cases (33 36) At present the chlorpromazine seems to be the best aid, but in no case should one fail to try different drugs which can have a rather variable influence on different symptoms in the carcinoid syndrome It also has to be pointed out that in one case (14) there has been reported a very good result from irradiation of the liver in a patient who had been proved to have liver metastases.

Summary

One case of endocrinely active bronchial carcinoid tumour without liver metastases in a 70-year-old man is related. Eight years after the removal of his primary tumour the patient presented typical symptoms of a functioning carcinoid. During the attacks he excreted up to 470 mg 5HIAA/24 h but even in a symptom-free interval he excreted up to 143 mg (normal value 2—10 mg/24 h)

The authors also present a survey of 26 cases with the same disease hitherto published, and the pathology biochemistry symptomatology diagnosis and treatment of a functioning bronchial carcinoid are discussed. In those cases where surgical therapy is impossible or has failed it is recommended to try besides symptomatic treatment, different substances which can affect the metabolism of serotonin.

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Chlorpromazine per os had no influence, but this substance given intravenously (150 mg/24 h) diminished the duration of the attacks to 24—36 hours and the patient was able to walk around during the attacks and the diarrhoea ceased. The frequency of the attacks was however the same.

Discussion

Until now 26 cases of bronchial adenomas with carcinoid syndrome have been reported. All of them have not been included in table I Buchberger (4) has related 13 cases of bronchial carcinoids, a great number of which had a carcinoid syndrome. Unfortunately these cases could not be arranged in our table. Mc Cormack et al. (18) have described some cases which presented "significant amounts of serotonin and were considered as functioning carcinoids However none of their cases revealed any clinical signs of hyperserotoninaemia.

In table I we can see that there is an equal sex distribution and that the age ranges from 31 to 72 years with a mean age of 48 years The carcinoid syndrome has made its first appearance up to 8 years after the operation which usually has been performed just after the discovery of the tumour There is marked variability in the frequency and duration of the attacks. The majority of the cases have two or more of the cardinal symptoms. The presence of pathologically increased amounts of 5HIAA in the urine and of flush is almost universal. There does not seem to be any relation between the severity of the disease and the amount of 5HIAA excreted Fully developed carcinoid syndromes with a fairly small elevation are reported (8, 12 17) On the other hand some patients have not had any symptoms in spite of a considerably increased excretion of 5HIAA Our own case had as mentioned before, in a symptom-free interval an excretion of up to 143 mg 5HIAA/24 h and Warner et al. (34) have recently reported 2 cases with hyperserotoniaemia and increased excretion of 5HIAA but without any sign or symptom of the carcinoid syndrome.

Positive evidence of endocardial fibrosis only in the right heart has, as far as we have found been reported in only 3 cases (1 5 37) Remarkably enough these cases had a rapid course and they were all dead within 2 years after the first symptoms. All of them had liver metastases. On the other hand in carcinoids in the intestine the endocardial fibrosis has been mentioned to be a late symptom and it is found there to a considerably greater extent (40 /)

As has already been stated, liver metastases are not essential for a carcinoid syndrome and this is illustrated by our own case.

The blood drainage of the bronchial carcinoids goes mainly to the left auricle and one would expect endocardial fibrosis chiefly in the left heart as the serotonin is eliminated mainly in liver and lungs However we have found in the literature only one example of this (15)

Other factors than a pure hyperserotoninaemia must therefore play a role in the origin of the carcinoid syndrome. It can be supposed that differences in haemodynamics and blood gases in the left and right heart may contribute to this. It is evidently difficult to explain alternatively the presence of an endocardial fibrosis in the right but not in the left heart, if we do not accept that the extraportal metastases can excrete considerably larger amounts of serotonin than the primary tumour Left-sided endocardial fibrosis also accompanies carcinoids in the intestine but only in combination with a

right-sided one. This corresponds better with expectation, and it can be assumed that the primary tumour and its extra portal metastases produce serotonin in amounts such that not all of it can be eliminated in the liver or the lungs.

In the first instance operation is recommended when possible. In cases where this is not possible or where there is a relapse after operation virtually nothing remains at present but symptomatic therapy With variable results ergotamine tartrate, noniacid, antihistaminic drugs, chlorpromazine, derivatives of rauwolfia serpentina and lysergic acid have been tried, all of them interfering with the metabolism of serotonin in different ways. Heparin is said to have a depressive effect on serotonin metabolism and has been tried with at least a temporarily good result (2) Alpha-methyldopa prevents the transformation of 5-hydroxy tryptophan to serotonin and has been effective to a certain degree in some few cases (33, 36) At present the chlorpromazine seems to be the best aid, but in no case should one fail to try different drugs which can have a rather variable influence on different symptoms in the carcinoid syndrome. It also has to be pointed out that in one case (14) there has been reported a very good result from irradiation of the liver in a patient who had been proved to have liver metastases.

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Acta Medica Scandinavica. Vol. 175, fasc. 1, 1964

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Correlation Between Histological Findings and Serum Transaminase Values in Chronic Diseases of the Liver

By

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Various enzymatic processes taking place under physiological conditions have been the subject of intensive study in recent years. Numerous enzymes are known. The most extensive practical application in diagnostics still pertains, however, to the glutamic-oxalacetic and glutamic-pyruvic transaminases. The part played by these enzymes in the diagnosis of acute hepatitis and the differential diagnosis of jaundice is well recognized (15, 19, 21, 22, 23). The activity of serum transaminase may indicate within certain limits whether the process in the liver has subsided after recovery from acute virus hepatitis (23, 24). Yet the significance of serum transaminase activity in chronic liver diseases is still not fully evident. According to the literature there are contradictions in the results and diagnostic value of these enzymes. Some authors have always met with normal results (4, 13). Others encountered part normal and part pathological values (2, 6, 15, 20, 24). Schmidt et al. (16) consider the enzymes in the serum as pathological for the duration of the inflammatory process in the histological findings in the liver. Baier et al. (1) discovered that increased serum transaminase levels were always accompanied by symptoms of a florid process in the liver, be it in chronic hepatitis or in cirrhosis. Normal values, on the contrary, do not exclude a florid chronic hepatitis or progressive cirrhosis.

The aim of the present study was to elucidate, on the basis of diagnoses confirmed by histological examination,

a) the activity of glutamic-oxalacetic and glutamic-pyruvic transaminases in chronic diseases of the liver,

b) the significance of these enzymes in judging the activity of the pathological process in the liver, and

c) the possibility that increased transaminase activity is linked exclusively to necrotic changes in the parenchymal cells of the liver.

Submitted for publication June 24, 1963.

Table I Serum transaminase levels in chronic diseases of the liver

	Cronic hepatitis	Cirrhosis	Steatosis	Posthep syndrome
Normal values	6	3	4	10
Increased values of SGOT & SGPT	8	7	2	0
Increased SGOT only	0	4	0	0
Increased SGPT only	11	2	4	0
Total	25	16	10	10

Material and methods

A total of 61 patients with chronic liver diseases were examined — 25 suffering from chronic hepatitis, 16 cirrhosis, 10 instances of fatty infiltration of the liver and 10 cases of posthepatitic syndrome. Comparison was made between histological findings and serum transaminase levels as well as other biochemical liver-function tests. The classification was established exclusively on the basis of histological analyses of material obtained by liver biopsy There is, of course, no clear-cut distinction between chronic hepatitis and cirrhosis of the liver the classification was based on the accepted pathologico-anatomical principles. Histological findings were normal in posthepatitic syndrome, the diagnosis being indicated by the clinical picture and laboratory findings (10)

The following biochemical analyses were performed

Serum glutamic-oxalacetic transaminase (SGOT) was estimated according to the method of U C. Dubach (3) Normal values range between 4 and 40 units.

For serum glutamic pyruvic transaminase (SGPT) the method of Wroblewski and Cabaud (23) was applied with normal values ranging from 1 to 45 units.

The determination of serum bilirubin was performed according to the Jandrassyk and Clegborn method (9) the upper normal limit is 1.2 mg %

The thymol turbidity test was carried out in accordance with the MacLagan method (14) by the Shank and Hoagland modification (17) normal values amount to 0 to 5 units.

The sublimate test was applied in accordance with the Keler and Devic (11) method normal values range from 0 to 5 units.

Alkaline phosphatase activity was estimated according to the Shinowara, Reinhardt and Jones modification (18) Normal values range from 2.2 to 5 B. units.

Total cholesterins were estimated by the Gringaut Fleury method (8) Normal values have a range of 150 to 200 mg %

The Vim-Silvermann method was utilized in performing liver biopsy The fragment of liver parenchyma obtained was fixed in 10 % formalin. The histological specimens were stained with hematoxylin-eosin, and for fat with sudan.

We endeavoured to estimate the activity of the pathological process by 1) the presence of regressive processes, especially necrosis of the liver cells 2) the extent and type of the inflammatory process 3) the proliferation and cellularity of the connective tissues and 4) the proliferation of bile ducts.

The severity of the pathological process was indicated by one to three crosses, the latter denoting the patho-histologically most active process.

Results

Transaminase activity

Table I presents the levels of serum glutamic-oxalacetic and glutamic pyruvic transaminase activity in chronic diseases of the liver

In 19 of a total of 25 patients with chronic hepatitis one or both transaminase values were increased similar conditions were observed in cirrhosis. The normal levels occurring in 6 patients with chronic hepatitis and 3 with cirrhosis indicate, however the lack of a strict correlation between the histological picture and serum transaminase activity As will be seen later the liver may be involved in a

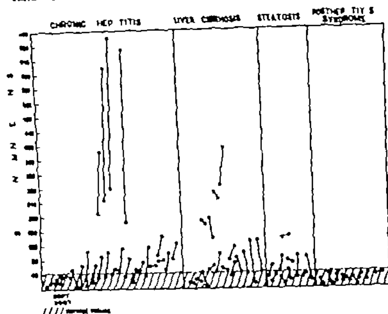


Fig 1 Transaminase values in chronic liver diseases

severe pathological process yet the transaminase values may be normal. Accordingly normal serum transaminase values do not warrant exclusion of a pathological process in the liver. Transaminase activity in fatty infiltration of the liver was at normal levels in four of ten patients, and elevated in the remaining six. A normal level of transaminase was seen in all our patients with posthepatitic syndrome, corresponding to the normal histological picture presented in all these cases.

In analysing the pathological levels of serum transaminase (fig 1) we find them moderately elevated in most cases of hepatitis ranging from slightly pathological values to 100 to 150 units. There was a very marked increase in 4 patients, in whom values as high as 500 to 900 units were reached. Serum transaminase activity takes a similar course in cirrhosis, yet it is slightly lower than in chronic hepatitis. A certain difference manifests itself in our material between the patients suffering from chronic hepatitis and those with cirrhosis, viz. in chronic hepatitis there is greater activity of glutamic pyruvic transaminase whereas in cirrhosis the glutamic-oxalacetic transaminase reaches higher activity in many patients. Transaminase values in 5 patients with fatty infiltration of the liver were but slightly increased (to 90 units). Only in one patient did glutamic-oxalacetic and glutamic-pyruvic transaminase activities rise to as high as 145 units.

The mean values and standard deviations of transaminase in the different groups of patients are given in table II. The difference in the mean values between normal subjects and patients with chronic hepatitis and cirrhosis is statistically significant ($p = < 0.05$). In fatty degeneration the results of SGOT activity are not statistically significant, the values of SGPT however are ($p = < 0.05$).

Table II Mean values and standard deviations in chronic hepatitis cirrhosis and steatosis

	Normal values		Chronic hepatitis		Cirrhosis		Steatosis	
	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
Mean values	21.5	20.4	69.3	167.5	110.9	98.1	48.2	69.6
S D (±)	12.4	13.6	76.5	237.8	86.08	104.04	48.1	39.7

Table III Correlation of transaminase level and activity of the pathological process in the liver

Transaminase level (units)	Diagnosis	No. of cases	Activity of histological findings		
			High	Moderate	Inactive
150	Chronic hepatitis	5	5		
	Cirrhosis	4	4		
	Steatosis	0			
From 45 to 150	Chronic hepatitis	14	8	2	4
	Cirrhosis	9	3	3	3
	Steatosis	6	1	4	1
Up to 45	Chronic hepatitis	6	3	3	
	Cirrhosis	3	1	1	1
	Steatosis	4			4

Table IV Correlation of transaminase activity and necrotic changes in the liver

Transaminase activity (units)	Diagnosis	No. of cases	Necrotic changes		
			++	+	0
150	Chronic hepatitis	5	1	1	3
	Cirrhosis	4	4	—	—
From 45 to 150	Chronic hepatitis	14	1	2	11
	Cirrhosis	9	5	—	4
Up to 45	Chronic hepatitis	6	2	—	4
	Cirrhosis	3	—		1

++ = minute patches of necrosis (in groups of 8 to 10 liver cells)
\+ = single liver cells necrotic,
0 = no necrotic liver cells present

Correlation of transaminase level and activity of the pathological process in the liver

A correlation was sought between transaminase values and the activity of the pathological process in the liver the patients being grouped according to the values — above 150 units (i e. markedly increased) 45 to 150 units, and normal (table III) The intensity of the inflammatory process in the periportal fields and the intralobular space, the appearance of the polygonal cells of the parenchyma, the proliferation of the bile ducts and in cirrhosis, the quantity of increased connective tissue were adopted as measures of the activity of the patholog

ical processes observable histologically The results of these analyses indicate the presence of a highly active process in the liver in all patients with transaminase values above 150 units. In 16 of 23 patients with a moderately increased transaminase activity the histological specimen showed signs of a moderate or highly active pathological process. In the others, the process in the liver was inactive. The third group, with normal values of transaminase, presents all transitory forms from an inactive to a highly active pathological process. Hence it follows from these results that high levels of transaminase activity indicate a highly active process in the liver in chronic hepatitis and cirrhosis. Normal or moderately increased values, however do not exclude a florid chronic hepatitis or progressive cirrhosis. In 7 patients with chronic hepatitis and cirrhosis there was moderately elevated transaminase activity although the histological findings indicated all the characteristics of an inactive process in the liver Hence a moderately increased transaminase activity need not always be a definite sign that the pathological process in the liver is florid. In fatty infiltration of the liver with normal transaminase activity there is either no inflammatory process or it is quite insignificant. Five patients with but slightly elevated transaminase values had signs of more or less marked concomitant inflammatory changes in the histological specimen. In one case the latter changes were lacking despite moderately increased transaminase values.

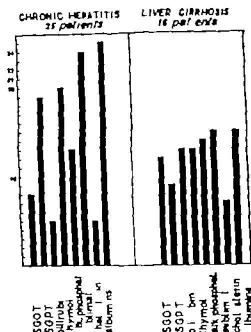


Fig. 2. Correlation of pathological values of biochemical analyses and values of transaminase

Comparison between transaminase activity and necrotic changes in the liver parenchyma

A correlation of transaminase activity values and necrotic changes in the liver is presented in table IV In the group of patients with transaminase values surpassing 150 units, 6 of 9 patients presented minute patches of necrosis or changes in individual cells only No degenerative changes were seen in 3 patients although transaminase activity was elevated. Necrotic and degenerative changes in the liver cells were absent in 15 of a total of 25 patients who had increased transaminase activity In contrast to this observation some patients were found to have minute patches of necrosis although their transaminase activity was normal. These results indicate a poor correlation between serum transaminase activity and the occurrence of necrobiosis of the liver cells.

Correlation of pathological values of biochemical tests and levels of transaminase activity

Pathological results yielded by biochemical tests carried out in cases of

chronic hepatitis and cirrhosis are presented on fig 2. Pathological conclusions were found most often for serum albumins and flocculation tests Transaminase activity was increased in a slightly lesser number of patients However in 7 patients with chronic hepatitis and 3 with cirrhosis an elevated level of transaminase activity was present although the flocculation tests especially the thymol turbidity test yielded normal or but slightly pathological values. Hence there is no complete correlation between transaminase activity and the other biochemical assays.

Discussion

Since the publication of the first reports by La Due et al. (12) on the increased activity of serum transaminase in myocardial infarction, the concentration of these enzymes in the serum has been investigated in a number of pathological conditions. The diagnostic significance of the activity of serum glutamic-oxalacetic and serum glutamic pyruvic transaminase in acute and chronic hepatic diseases was studied by numerous authors, as the liver parenchyma is rich in transaminases. The serum of patients with acute hepatitis always presents very high values of transaminase activity In chronic hepatitis and cirrhosis, on the contrary transaminase activity is increased in only some of the patients. No strict correlation hence prevails between histological findings and transaminase activity consequently normal levels of transaminase do not preclude the presence of chronic hepatitis and cirrhosis. Transaminase activity is but moderately elevated in most patients with pathological levels. Very high levels are observed in exceptional instances only

There is a certain differrence in the mean levels of transaminase activity between chronic hepatitis and liver cirrhosis. Serum glutamic pyruvic transaminase is more often increased in chronic hepatitis while the activity of serum glutamic-oxalacetic transaminase reaches higher levels in cirrhosis. Despite this difference occurring in certain cases, the determination of the SGOT and SGPT quotient cannot be utilized in the differential diagnosis of chronic hepatitis and cirrhosis. An increased activity of serum glutamic-oxalacetic transaminase as compared to serum glutamic pyruvic transaminase in cirrhosis has been established by numerous other authors (1 19 20 22) This occurrence may be connected with the increased concentration of glutamic-oxalacetic transaminase in the liver parenchyma in cirrhosis as compared to the control group although there is no definite strict correlation between the quantity of the enzyme within the liver parenchyma and the serum transaminase activity (19 20) The reason for a rather frequent occurrence of increased glutamic pyruvic transaminase activity in chronic hepatitis is not clear This difference points to the complicated regulating mechanism responsible for the production and elimination of enzymes from the cytoplasm of the cell of the liver parenchyma

An increased activity of glutamic-oxalacetic and glutamic pyruvic transaminase often signals activity of the process in the liver in case of chronic hepatitis and cirrhosis. An accurate correlation between the activity of the process in the liver and the level of transaminase activity was observed only in patients with markedly elevated levels of serum transaminase (above 150 units) Normal or moderately increased values do not ex

clude a florid chronic hepatitis or progressive cirrhosis.

Transaminase values may be moderately elevated in some patients even though the histological findings in the liver are inactive. Such findings indicate that the intracellular metabolism in the liver parenchyma may be disturbed even with no histological symptoms of activity of the process in the liver.

A lack of strict correlation between the level of transaminase and the activity of the process in the liver has been evident also in patients in whom several subsequent biopsy examinations were performed at 2 months intervals. Although the transaminase activity was reduced from markedly increased levels to normal the histological specimen still exhibited all the characteristics of an active hepatitis.

No complete confirmation was established for the opinion of numerous authors (2, 5, 15, 24) who connect the increased transaminase activity with a liberating of these enzymes from the necrotically changed liver cells. As deduced from our results, in some patients with markedly increased and with moderately increased transaminase levels there were no signs of necrosis of the liver cells in the histological specimen (obtained by means of biopsy) although other histological signs of an active process were present. Accordingly the level of transaminase does not run parallel with the degree of lesion of the liver cells hence it is impossible to determine the proportion of damaged cells from these findings. Most probably the serum transaminase levels in chronic hepatitis and cirrhosis partly depend on an increased permeability of the cell membrane, in the inflamed tissues (7). In rarer instances the cell membrane may be permeable although the inflammatory process is inconspicuous, probably due to a disturbed intracellular metabolism.

The mean values of transaminase activity are somewhat lower in fatty infiltration of the liver than they are in chronic hepatitis and cirrhosis. Elevated levels of transaminase in the course of steatosis usually indicate the presence of reactive concomitant inflammatory changes which probably increase the permeability of the cell membranes. However the elevated levels of transaminase in one of our patients with pure steatosis, and similar findings of other authors (1) indicate the possibility that a disordered intracellular lipid metabolism — even without concomitant inflammatory changes — may lead in some patients to an increased transaminase activity.

Summary and conclusion

The correlation between histological findings and serum transaminase activity was investigated in 25 patients with chronic hepatitis and 16 with liver cirrhosis, 10 cases of fatty infiltration of the liver and 10 patients with posthepatitic syndrome. One or both transaminase activities are elevated in the majority of patients suffering from chronic hepatitis and cirrhosis. The normal levels of transaminase activity found in one patient out of every four with chronic hepatitis and one out of five with cirrhosis indicate a lack of strict correlation between histological findings and serum transaminase activity. Hence normal transaminase values do not preclude the presence of a pathological process in the liver.

In comparing transaminase levels with the activity of the pathological process in the liver in chronic hepatitis and cirrhosis,

we found that only very elevated levels of transaminase activity (above 150 units) indicate with some certitude the presence of an active process in the liver Normal or moderately raised levels, however do not preclude a florid hepatitis or progressive cirrhosis In contrast to the above, transaminase activity was moderately elevated in some patients although the histological findings exhibited all the characteristics of an inactive process in the liver Thus a moderate rise in the level of transaminase activity does not prove activity of the pathological process in the liver

Normal transaminase activities in fatty infiltration point to pure steatosis Elevated levels are usually found in the presence of concomitant inflammatory changes.

Elevated transaminase activity in chronic hepatitis and cirrhosis need not arise from necrobiosis of the cells. Permeability of the cell membrane in tissue which is undergoing inflammatory changes or in consequence of disturbed intracellular metabolism often leads to rises in transaminase levels

The estimation of transaminase activity in chronic hepatic diseases has some value as an accessory laboratory method, but its usefulness should not be overestimated.

Acknowledgement

Miss Gizela Lukovic, Assistant, School of Public Health, gave valuable assistance in th statistical analysis.

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Acta Medica Scandinavica. Vol. 175 fasc. 1 1964

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Transseptal Left Heart Catheterization with Puncture of the Interatrial Septum

By

O. Lindeneg, M. H. Nielsen and A. Tybjærg Hansen

During the last 10 years, several methods of determining the haemodynamic conditions in the left heart have been developed. However all these methods have certain limitations; they supplement each other to a certain extent but do not furnish sufficient diagnostic data in all cases. The aorta and the left ventricle can be explored by arterial catheterization, but the conditions of the left atrium and the pulmonary veins cannot be elucidated by this method. The various percutaneous punctures (4 11 14) and transbronchial puncture (9) of the central blood vessels and the heart are well suited for pressure recordings, but they permit of other examinations to limited extent only.

During recent years, a method has been employed in many centres (3 7 15) by which the possibilities of exploring the left heart are improved. The technique consists in puncture of the left atrium through the interatrial septum by means of a needle which is introduced into the right atrium through a peripheral vein, and in a subsequent left heart catheterization through the puncture site in the interatrial septum. Shunt determinations with inert gases (3 6, 17 20) and inspection of the interatrial septum during open heart surgery (1 8) have shown that no permanent atrial septal defect results from puncture of the septum.

The method has now been adopted and further developed in many centres (1 5 6, 8, 19 20) and it has given good results with but few complications.

We have employed the methods developed by Ross (16 18) and Johnson (10) and modified the technique, of which a brief description is given below.

Technique and procedure

Prior to the examination, normal bleeding time and normal coagulability of blood have been ascertained. The patients are premedicated with phenobarbital 2 hours prior to the examination, and at the beginning of the examination the area around the femoral vein is infiltrated with 1 solution of xylocaine-adrenaline®.

The femoral vein is punctured with needle 12 cm in length with an outer diameter of

Submitted for publication June 28, 1963.

Table I Diagnosis and results in transseptal heart catheterization in 64 cases

Diagnosis	No of invest.	Left heart not examined	Diagnostic data obtained during transseptal catheterization	
			Adequate	Inadequate
Mitral valvular disease	21	7	6	8
Aortic valvular disease	27	3	16	8
Mitral + aortic valvular disease	3	—	1	2
Pulmonary stenosis	2	—	2	—
Cor pulmonale	1	—	1	—
Abnormal pulmonary veins	1	—	1	—
Coarctation of the aorta	1	—	1	—
Patent ductus arteriosus	2	2	—	—
Abnormal rotation of the heart	2	2	—	—
Normal haemodynamic findings	4	—	4	—
Total	64	14	32	18

2 mm and an internal diameter of 1 5 mm. A No. 4 Cournand catheter with a steel guide is introduced into the vein by way of the needle. After the needle is removed the larger catheter is inserted in the vein, the Cournand catheter with steel guide serving as a stylet. The right heart is catheterized by means of a No 7 Lehmann catheter the Teflon catheters used in left heart catheterization being too stiff and with an inappropriate curve.

Puncture of the interatrial septum is performed by means of the needle described by Ross, with an outer diameter of 1 4 mm and 61 cm in length, or by means of a needle measuring 1.2 mm in diameter and 82 cm in length (supplied by Dick, Copenhagen) The needles are curved differently at the tip, adapted to the size of the right atrium. When the right heart catheterization is completed the Lehmann catheter is replaced by a No. 8 Teflon catheter and the right atrium is catheterized. The puncture needle is inserted into the Teflon catheter the needl being fitted with a blunt steel stylet, which projects 5 mm outside the tip of the needle in order to prevent damage to the catheter When the puncture needle has been advanced as far as 5 cm from the tip the catheter and the needle are rotated so that the tip faces towards the left and 45 backwards. The catheter and the needle are now pushed towards the inter atrial septum a little below the centre of the atrial shadow When the catheter is in its correct position, its tip will be caught by the limbus fossae ovalis, and when the catheter is pushed further on, it will curve cranially The needle is then advanced to the septum, the stylet is removed, and the needle is connected to a manometer The septum is punctured and we made sure of the correct position of the needle in the left atrium by taking a pressure recording (fig. 1) The Teflon catheter is now pushed forwards, the needle is removed, and left heart catheterization is performed (fig 6)

The Teflon catheters employed are radiopaque, produced from Teflon tubing (U S. C. I) or delivered ready for use (Polystan, Copenhagen) To facilitate catheterization of the left ventricle and the aorta, the Teflon catheter is curved 360 over the distal 15 cm.

When simultaneous pressure readings are taken, a radiopaque catheter No. 4 is inserted through the wider catheter and each of the catheters is connected to a manometer by means of a forked metal tube one branch of which is furnished with a stopcock, the other with a firm rubber gasket.

Radiopaque dye is injected through catheters with *side holes*, while the *hole at the* tip of the catheter is closed by means of an obturator consisting of a metal ball on a thin steel wire. The wire is fixed outside the lateral tube of the stopcock.

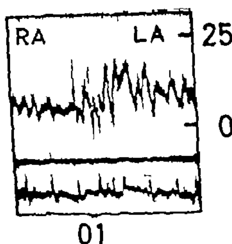


Fig. 1. Pressure readings during interatrial septal puncture. Patient with mild degree of aortic stenosis. RA right atrium, LA left atrium.

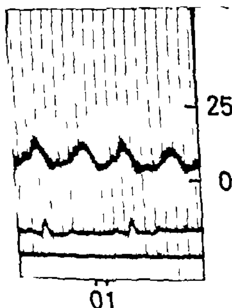


Fig. 2. Simultaneous pressure readings in the left atrium through the internal and the external catheters. Patient with mild degree of aortic stenosis.

Material

Over the period from December 1960 to July 1962, attempts at transseptal cardiac catheterization were made in 64 cases. The

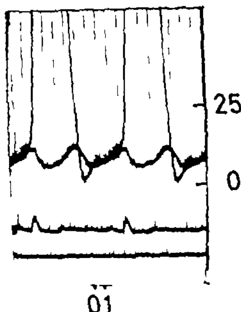


Fig. 3. Simultaneous pressure readings in the left atrium through the external catheter and in the left ventricle through the internal catheter. Patient with mild degree of aortic stenosis.

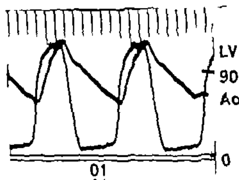


Fig. 4. Simultaneous pressure readings in the left ventricle and the aorta in patient with aortic incompetency. AO aorta, LV left ventricle.

examination was successful in 50 cases (78 %) while catheterization failed in 14 cases (table I). In 2 out of these 14 patients, we did not succeed in entering the femoral vein, and in 4 patients it was impossible to push the needle through the iliac vein or the inferior caval vein. In 2 patients with abnormally rotated hearts, the fossa ovalis could not be localized,

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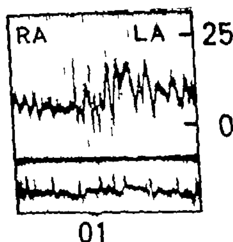


Fig. 1 Pressure readings during interatrial septal puncture Patient with mild degree of aortic stenosis RA right atrium, LA left atrium.

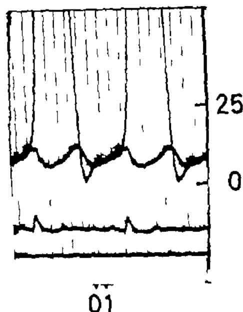


Fig. 3 Simultaneous pressure readings in the left atrium through the external catheter and in the left ventricle through the internal catheter Patient with mild degree of aortic stenosis.

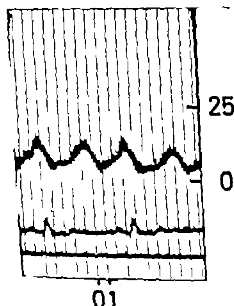


Fig. 2. Simultaneous pressure readings in the left atrium through the internal and the external catheters Patient with mild degree of aortic stenosis

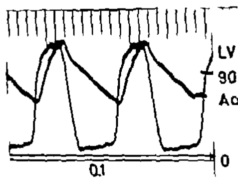


Fig 4 Simultaneous pressure readings in the left ventricle and the aorta in patient with aortic incompetency AO aorta, LV left ventricle.

Material

Over the period from December 1960 to July 1961, attempts at transseptal cardiac catheterization were made in 64 cases. The examination was successful in 50 cases (78) while catheterization failed in 14 cases (table 1) In 2 out of these 14 patients, we did not succeed in entering the femoral vein, and in 4 patients it was impossible to push the needle through the iliac vein or the inferior caval vein. In 2 patients with abnormally rotated hearts, the fossa ovalis could not be localized,

Table I. Diagnosis and results in transseptal heart catheterization in 64 cases

Diagnosis	No. of invest.	Left heart not examined	Diagnostic data obtained during transseptal catheterization	
			Adequate	Inadequate
Mitral valvular disease	21	7	6	8
Aortic valvular disease	27	3	16	8
Mitral + aortic valvular disease	3	—	1	2
Pulmonary stenosis	2	—	2	—
Cor pulmonale	1	—	1	—
Abnormal pulmonary veins	1	—	1	—
Coarctation of the aorta	1	—	1	—
Patent ductus arteriosus	2	2	—	—
Abnormal rotation of the heart	2	2	—	—
Normal haemodynamic findings	4	—	4	—
Total	64	14	32	18

2 mm and an internal diameter of 1.5 mm. A No. 4 Cournand catheter with a steel guide is introduced into the vein by way of the needle. After the needle is removed the larger catheter is inserted in the vein, the Cournand catheter with steel guide serving as a stylet. The right heart is catheterized by means of a No. 7 Lehmann catheter, the Teflon catheters used in left heart catheterization being too stiff and with an inappropriate curve.

Puncture of the interatrial septum is performed by means of the needle described by Ross, with an outer diameter of 1.4 mm and 61 cm in length, or by means of a needle measuring 1.2 mm in diameter and 82 cm in length (supplied by Dick, Copenhagen). The needles are curved differently at the tip, adapted to the size of the right atrium. When the right heart catheterization is completed, the Lehmann catheter is replaced by a No. 8 Teflon catheter and the right atrium is catheterized. The puncture needle is inserted into the Teflon catheter, the needle being fitted with a blunt steel stylet, which projects 3 mm outside the tip of the needle in order to prevent damage to the catheter. When the puncture needle has been advanced as far as 5 cm from the tip, the catheter and the needle are rotated so that the tip faces towards the left and 45° backwards. The catheter and the needle are now pushed towards the interatrial septum a little below the centre of the atrial shadow. When the catheter is in its correct position, its tip will be caught by the limbus fossae ovalis, and when the catheter is pushed further on, it will curve cranially. The needle is then advanced to the septum, the stylet is removed, and the needle is connected to a manometer. The septum is punctured, and we made sure of the correct position of the needle in the left atrium by taking a pressure recording (fig. 1). The Teflon catheter is now pushed forwards, the needle is removed, and left heart catheterization is performed (fig. 6).

The Teflon catheters employed are radiopaque, produced from Teflon tubing (U. S. C. I.) or delivered ready for use (Polystan, Copenhagen). To facilitate catheterization of the left ventricle and the aorta, the Teflon catheter is curved 360° over the distal 15 cm.

When simultaneous pressure readings are taken, a radiopaque catheter No. 4 is inserted through the wider catheter and each of the catheters is connected to a manometer by means of a forked metal tube one branch of which is furnished with a stopcock, the other with a firm rubber gasket.

Radiopaque dye is injected through catheters with side holes, while the hole at the tip of the catheter is closed by means of an obturator consisting of a metal ball on a thin steel wire. The wire is fixed outside the lateral tube of the stopcock.

cases into the left atrium and in 15 cases into the left ventricle (figs. 8, 9 and 10)

In 6 cases left valvular disorder was evaluated by means of simultaneous pressure readings through the internal and the external catheters on either side of the affected valves at rest and on exercise, together with determination of the cardiac output (figs. 2, 3 4, and 5)

Complications

The great majority of the patients of the present series suffered from severe and advanced cardiac diseases. Just as this circumstance gave rise to an increase in the number of unsuccessful punctures, the complications are also encountered primarily in the more seriously ill patients. In 4 patients atrial fibrillation occurred during the initial right heart catheterization, in one case the arrhythmia stopped simultaneously with the accomplishment of the puncture of the inter atrial septum. Atrial fibrillation never occurred in connection with or after puncture.

In 2 patients sinus arrest or sinoatrial block developed. In one of these patients the arrythmia occurred before any attempt at septal puncture was made, the catheter and the puncture needle still being situated in the right atrium. In the other patient the septal puncture had been accomplished, but the catheter choked up and was, therefore, withdrawn into the right atrium. Immediately afterwards the arrhythmia started. In both patients the arrhythmia ceased quickly in one case after administration of atropine, in the other without any treatment.

Three patients with rheumatic valvular disorders and severe dyspnoea on exertion had for several years had angina pectoris. On attempts at puncture they got typical precordial pains and in one of them the puncture had to be abandoned. The pains subsided spontaneously in the two patients suffering from mitral disorder while in the third, who had combined mitral and aortic stenosis, fall in blood pressure and bradycardia developed. The patient recovered in the course of few hours following treatment with atropine, Aramine® and morphine.

In one patient with an abnormally rotated heart, the posterior wall of the atrium was



Fig 9 Left ventriculography in patient with valvular aortic stenosis. Dome-shaped valves are seen during the systole Frontal projection.



Fig 10. Left ventriculography in the same patient as fig 9 Lateral projection.

punctured. X-ray examination immediately afterwards revealed small localized infiltration, which disappeared in the course of one week.

Shortly after puncture of the interatrial septum, one patient suffering from cor pul-

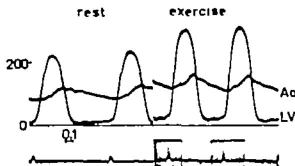


Fig 5 Simultaneous pressure readings in the inflow tract of the left ventricle and in the aorta at rest and on exercise in a patient with severe fibrous subval ular aortic stenosis. AO aorta, LV left ventricle



Fig 6 The catheter being passed through the interatrial septum, the left trium, the left entricl and out into the aorta, in a patient in whom the outflow tract of the left ventricle and the orta are positioned anteriorly and t the left.



Fig 7 Selective angiocardiography in the pulmonary vein in a patient with stenosis of the pulmonary vein, opening into the left trium.



Fig 8 Left ventriculography in a patient with mitral competency Pronounced regurgitation of the radiopaque dye int the enlarged left trium is seen

and n 3 patients the right atrium was too large so that the needle could not be brought into contact with the interatrial septum Twice the investigation was interrupted on account of arrhythmia and once because of angina pectoris.

Out of 50 cases of transseptal catheterization, the examination rendered adequate diagnostic information in 32 cases. In another 8 cases the examination could immediately be combined with retrograde catheterization of aorta or percutaneous puncture, and hereby a total of 40 patients or 63 of the entire material was examined adequately In 4 patients, the catheter was passed without puncture from the right to the left atrium by way of a patent foramen ovale.

Selective angiocardiography was performed in 18 cases. In one case the contrast was njected into the pulmonary veins (fig 7) n two

The complications of transeptal heart catheterization are but few. In a number of reports comprising a total of 431 investigations, death in connection with the investigation is reported in one case (20). Thus the transeptal catheterization is one of the safest methods of left heart catheterization (11). As regards sever complications, moreover, puncture of the ascending aorta from the right atrium was reported in 4 patients (6, 8, 19). Two of these developed haemopericardium which required aspiration. In two cases blood in the pericardium was unexpectedly found at subsequent surgery, indicating a puncture of the atrial wall (19).

We have punctured the posterior wall of the right atrium in one case, and got evidence of bleeding at X-ray examination. The most frequent complication in our series is disturbance of the transmission of cardiac impulses. The conduction disturbances are the same as those encountered in right heart catheterization, but in our series their frequency is much higher.

Summary

The technique of transeptal heart catheterization modified by the authors is described. Results and complications from 64 investigations are reported, and on this basis the indications for transeptal heart catheterization are discussed.

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monale developed prolonged hypotension. Prior to the puncture, right heart catheterization had been performed and following the puncture the aorta was catheterized from the femoral artery One hour after the septal puncture the patient developed shock in connection with puncturing of a peripheral artery The condition did not become stable till 48 hours later following treatment with noradrenaline.

In the case in which angiocardiography of the pulmonary veins was performed, dye was injected through a catheter without sideholes, and extravascular deposit occurred. This patient had blood-streaked sputum during the following 24 hours.

In one case a late complication was seen. This patient had a mitral stenosis, and 36 hours after an otherwise uncomplicated septal puncture she developed a transitory right hemiparesis.

Discussion

During recent years, many authors advocated the transseptal heart catheterization as the most advantageous method of exploring the left heart. According to our experiences from transseptal heart catheterization direct punctures of the great vessels and the heart (11) and arterial catheterization each of these methods presents advantages which are decisive in the selection of the proper method in each individual examination

In all cases of heart diseases in which the diagnosis cannot with certainty be established by right heart catheterization we prefer the transseptal catheterization which makes possible an examination of the left heart including angiocardiography and phonocardiography (2) When catheterization is performed from the femoral vein the right and left heart can be examined in a single session

Like other authors (8, 17 20) we find that only transseptal catheterization permits prolonged haemodynamic observations on the left heart under varied conditions. Transseptal catheterization is the best method of investigating the pulmonary circulation in the cases where it is desired to withdraw blood samples or to inject contrast medium in the pulmonary veins

In cardiac disorders localized in the left atrium, the choice of the method of investigation depends on the nature of the disorder Thus we prefer the transseptal catheterization in mitral incompetency and cor triatriatum, but percutaneous puncture in mitral stenosis, in which case it is difficult to catheterize the left ventricle from the left atrium. In case of tumours in the left atrium, angiocardiography in the pulmonary artery will often be sufficient to establish the diagnosis or otherwise instructive as to the choice of further investigations.

In some cases, clinically definite anomalies of the left ventricle and the aorta can be evaluated sufficiently by means o the transseptal catheterization, but generally the percutaneous punctures and the arterial catheterization give just as good or better results.

In cases with abnormally rotated hearts or heavily enlarged right atrium, in which cases the puncture needle cannot be placed in a safe position against the fossa ovalis, puncture of the interatrial septum should hardly be attempted.

Our experiences from interatrial septal punctures in patients with angina pectoris are limited but tend to show that the examination is but poorly tolerated The three patients examined got typical pains in connection with the septal puncture, and one developed shock. The only death reported in association with interatrial septal puncture occurred in a patient with coronary sclerosis (20)

Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Shock in Acute Myocardial Infarction

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A generally accepted definition of "shock" is not available, but there is a consensus of opinion that a number of non-specific symptoms are usually involved including tachycardia, pallor lowered skin temperature, oliguria and sensory disturbances although all of these symptoms need not be present at one time in the individual case. Provoking factors are numerous, but even so the compensatory measures instituted during the state of shock are usually uniform.

In cases of infarction the development of shock represents a well-known and dreaded complication. Although the therapeutical principles admittedly vary much most authors have found the death rate to range between 70 and 100 per cent, at least in studies based on rigid criteria (6, 32

The highly divergent interpretations of therapies are reflected primarily in the fact that several aspects of the mechanism of cardiogenic shock still remain to be elucidated. Studies on haemodynamics from recent years — in man as well as in experimental animals — have contributed greatly to our understanding of this mechanism and hence the introduction of a more rational therapy cannot be far away. Besides, many of the procedures which until now have been used only in animal experiments may in the not too far future be introduced into the clinical routine.

Since interest in this syndrome is probably increasing we have decided to report some clinical data on patients with myocardial infarction emphasizing in particular the development of shock.

Pathophysiology

The initial and fundamental disturbance in cardiogenic shock is represented by the sudden fall in stroke volume attributable to deficient power of contraction of the infarcted myocardium (8, 12, 15 21 22) In addition also the action of the remaining "non-affected" myocardium may be reduced as the injured areas actually expand during systole (10 36)

Although an attempt is made to compensate for the lowered stroke volume and the reduced cardiac output by a rise in the ventricular filling pressure and the onset of tachycardia, and is most probably

Submitted for publication June 28, 1963

Table 1. Distribution according to age and sex together with incidence of shock in 354 patients with acute myocardial infarction

Age groups (yrs)	Total no. of men	Men with shock	%	Total no. of women	Women with shock	%
<40	2	0	—	0	0	—
40–49	28	1	3.6	7	2	—
50–59	71	9	12.7	12	0	—
60–69	76	9	11.8	37	3	8.1
70–79	64	5	7.8	41	3	7.3
≥80	26	0	—	10	2	—
Total	247	24	9.7	107	10	9.3

emphasized that coronary shock has been seen to develop after bilateral cervical vagotomy. In experiments with animals in shock the authors determined the action potential of the cut cervical vagus in the upper dorsal roots, and in the splanchnic nerves they arrived at the opinion that the upper dorsal posterior sympathetic roots might be serving as afferent pathway. This theory has since gained some clinical support and will be laborated later.

Material

Three hundred and fifty-four patients are included in the present material, i.e. most of the patients admitted to the Municipal Hospital in Copenhagen, Department VII in the years 1945 to 1963, the diagnosis was in all cases one of cardiac infarction. Cases are included only if the diagnosis had been verified by electrocardiography or by autopsy findings.

The finding of systolic blood pressure values equalling or below 90 mm Hg in patients presenting acute coronary occlusion has formed the basis on which shock is defined, irrespective of the presence of clinically manifest symptoms of shock.

The 354 patients include 247 men and 107 women. In conformity with the above definition shock was encountered in 24 men and 10 women, totalling 34 patients.

Results

Age and sex together with incidence of shock

In the two main groups — patients with and without shock — the classification according to sex was identical, the ratio of men to women being 2.4 : 1 and 2.3 : 1. In the material discussed in this paper the distribution into age groups of men and women was as follows (table 1): 40 to 49 years 4 : 1, 50 to 59 years 5.9 : 1, 60 to 69 years 2.1 : 1, 70 to 79 years 1.6 : 1, 80 years and above 0.6 : 1, thus the present study shows the well known age variations (18, 37).

The incidence of shock was the same in men and women (9.7 and 9.3 %), although the diagnosis is based on blood pressure values exclusively with no regard to accompanying clinical symptoms of shock, figures here reported are not higher than those found by previous investigators basing their studies on more rigid criteria. Binder et al. (6) reviewed nine comprehensive surveys, including a total of 650 patients with infarction, always discriminating between studies on "low incidence" and "high incidence". In the former category shock was encountered in about 12 per cent, in the latter in about 45 per cent of the cases.

ascribable to the falling blood pressure via the receptors in the sinus and the aortic arch the general outcome will be reduced cardiac output. In his review on the matter Friedberg (14) states that the cardiac output in cases of cardiogenic shock is not infrequently seen to be reduced by 25 to 80 per cent and that symptoms of shock generally develop in patients in whom the cardiac output is most markedly reduced But indeed other symptoms apart from the reduction in cardiac output seem to be involved a feature which is reflected in the fact that some of the patients presenting a much reduced cardiac output escaped the development of shock.

Consider the ratio of mean arterial blood pressure (MAP) to cardiac output (CO) and peripheral resistance (R)

$$MAP = CO \times R$$

and it will be seen that a fall in blood pressure to values characteristic of shock is not possible unless the reduced cardiac output is not compensated by sufficiently intensified peripheral resistance.

No doubt the clinical features of shock conditions may have given rise to the assumption that it was a matter of a maximal vasoconstriction originating in the sympathetic nervous system thus explaining the clinical manifestation but not the fall in blood pressure which by adequate distribution of the circulating blood volume served as a kind of protection

Recent studies, however have modified this opinion. Agress et al (1) provoked cardiac infarction in dogs by sending emboli of "plastic microspheres into the coronary arteries. Shock (40 fall in blood pressure) was seen to develop in some of the animals, namely in those in which the peripheral resistance remained uninfluenced or was only slightly increased. The favourable results obtained since the introduction of pressor substances show also that shock is not attributable to an existing intensive vasoconstriction but rather develops if the peripheral resistance fails to increase sufficiently

If compensatory measures fail to raise the blood pressure the outcome will be a reduced coronary flow The curtailment of diastole caused by the tachycardia will act in the same direction and the cardiac output will be further reduced In cases of protracted shock chances of survival lessen because of various complications such as congestive heart failure, fluid retention syndrome ("lower nephron nephrosis") or symptoms attributable to a deficient cerebral blood supply including respiratory arrest due to failure of the respiratory centre or a release of the venoconstrictor tone involving a diminution of the ventricular filling pressure, the sequelae of which are represented by a further reduced cardiac output and falling blood pressure (30)

Numerous explanations have been offered to elucidate why the peripheral resistance is not sufficiently increased in all cases. Von Euler (12) suggests that a "local lack of oxygen in the vascular walls and tissue is responsible for an ineffective reaction to pressor substances according to de Lee (22) vasodilating substances may have been liberated from the infarct or the surrounding ischaemic areas.

Finally the normal haemostasis also may be altered because of reflexes from the injured myocardial area or due to some hormonal factor According to Schimert (31) it is the Jarish-Bezold reflex which is responsible for the hypotension during shock but this contention is challenged by Agress et al. (1) who

Table IV Localization of infarct

	Total no.	Anterior and posterior walls		Anterior wall		Posterior wall	
		No.		No.		No.	%
Men							
Without shock	223	11	4.9	125	56.0	87	39.1
With shock	24	1	4.2	10	41.7	13	54.1
Women							
Without shock	96	6	6.2	44	45.9	46	47.9
With shock	10	0		6		4	
Total no. of pat.							
Without shock	319	17	5.3	169	52.9	133	41.8
With shock	34	1	2.9	16	47.1	17	50.0
Total	353	18	5.1	185	52.4	150	42.5

the initial 24 hours following the occlusion about 70 were admitted within 2 days about 12 were admitted more than one week after the occlusion had occurred.

Cardiac condition at time f admission

The blood pressure alues prior to the infarction ar known only in 64 of the cases. Twenty-two presented normotensive values BP equalling or below 160/110 mm Hg) shock developed in two of these 9 1) Fourty-two of the patients were suffering from hypertension shock developed in 6 of these cases (14.3) The presence or absence of heart symptoms prior to the occlusion is known however in 336 of the 354 patients (table III

In the column "heart failur " patients are recorded whom symptoms of heart failure had been diagnosed on previous occasions together with patients who became dyspnoeic on exertion or at rest, or were prone to oedema and no extra-cardiac explanation seemed satisfactory But the medical records failed to provide a basis on which to decide definitely whether these previous symptoms originated in cardiac disorders and hence the percentages should be taken with a certain reservation. In the column an gina pectoris cases are recorded in which attacks of pain affecting the precordium had occurred more than one month be fore the infarction although we ha e not been able to obtain further details such as the release of such pai ts radiation, response to nitrites, etc. The column previous infarction" includes cases in which basis was provided for the establishment of such diagnosis, either by electrocardiography or by findings at autopsy together with cases of which data from previous hospitalizations were available The percentage of previous infarction range probably bove the percentage given here 8 3 (Doscher and Poindexter (9) found 12.7 Ly ager Nielsen (23) found 14.9)

Table III shows otherwise unambig uously that cardiac infarction — partic ularly in women — is prone to develop

Table II Time of admission

	Intervals between onset of occlusion and admission			
	< 24 hours	1—2 days	3—7 days	> 1 week
Men	147	30	40	30
Women	61	13	19	14
Total	208 (58.7 %)	43 (12.2 %)	59 (16.7)	44 (12.4 .)

Table III Cardiac condition prior to admission for acute coronary occlusion

	No. of pat.	No heart symptoms		Heart failure		Angina pectoris		Previous history of infarction	
		No.	%	No.	%	No.	%	No.	.
Men									
Without shock	214	90	42.1	68	31 7	102	47 6	18	8.4
With shock	21	8	38.1	9	42.8	7	33.3	3	14.3
Women									
Without shock	92	16	17 4	52	56.5	54	58.7	6	6.5
With shock	9	3		4		5		1	
T tal no of pat									
Without shock	306	106	34.6	120	39.2	156	50.9	4	7.8
With shock	30	11	36.6	13	43.3	12	40.0	4	13.3
Total	336	117	34.8	133	39 6	168	50.0	28	8.3

Malach and Rosenberg (25) found manifestations of shock in 9 4 per cent of 264 patients with infarction and suggested that the grave prognosis in such cases was ascribable in part to the advanced age of the patients, 20 out of 25 being above the age of 60 years In our material the incidence of shock was almost identical in the age groups 50 to 60 years and 60 to 70 years The small number of patients who were younger than 40 years and older than 80 does not allow reliable conclusions to be drawn here.

Time of occurrence of shock

The large majority of the cases of shock became manifest less than 24 hours after the occlusion had occurred this applies to 25 of the 34 cases in 3 of the cases the shock developed after intervals of 1 to 3 days in 4 of the cases after intervals of 3 to 7 days In 2 cases the interval was more than one week.

Interval between occlusion and admission to hospital

It will be noted from table II that about 60 of the patients were admitted within

admission			Rhythm at admission			
80—100	> 100	No data	Sinus rhythm	Atrial fibrillation	Ventricular/ supra-ventricular tachycardia	No data
9 (32.9%)	7 (41.2%)	0	14 (82.4%)	1 (5.8%)	1 (5.8%)	1 (5.8%)
0	1	0	1	0	0	0
67 (39.6)	45 (26.6)	3 (1.9%)	160 (94.7%)	6 (3.5%)	1 (0.6%)	2 (1.2%)
3 (18.7%)	9 (56.3%)	0	13 (81.3)	0	2 (12.5%)	1 (6.2%)
37 (27.8)	40 (30.1%)	0	117 (88.0%)	12 (9.0%)	0	4 (3.0%)
4 (23.6%)	5 (28.8)	2 (11.7%)	15 (88.3)	0	0	2 (11.7%)
113 (35.3)	92 (29.2%)	3 (0.9%)	291 (91.2%)	19 (5.9%)	2 (0.6)	7 (2.3)
7 (20.6)	15 (44.2)	2 (5.8%)	29 (85.3%)	0	2 (5.8%)	3 (8.8%)

Condition of patients at time of admission

Whether the shock had developed early or late after the occlusion almost all of the patients were in shock when they arrived in hospital at which stage only two of the total number of 34 patients presented normal blood pressure values. This feature should be borne in mind when the grave prognosis is considered. Most of the patients without shock were found to be normotensive (70) whereas blood pressure values above 160/110 were found in about 27 (table V).

According to expectation the pulse rate was higher in patients with shock than in those in whom this complication did not develop although values below 80/min. were recorded in about 29.4 % of the cases in the former group. Compensatory tachycardia was a particularly rare finding in patients with shock and infarction affecting the posterior wall, probably because of the higher incidence in this group of atrioventricular blocks. It should be taken into consideration, however, whether the reflexogenic compensatory power of the organism, if any, is most severely damaged by lesions affecting this site of the myocardium.

On the basis of the initial ECG most of the patients were found to have sinus rhythm. Atrial fibrillation was never recorded in patients with shock and represented a rare finding in the other patients (5.9 %). Supraventricular and ventricular tachycardia were seen almost exclusively in patients with shock (5.8 % as against 0.6) and always in cases in which the infarction affected the anterior wall.

Condition of patients during hospitalization

It has been mentioned that many of the patients presented symptoms of heart failure and quite often the condition was seen to aggravate in continuation of the infarction. In 20—30 % of the cases the symptom developed *after* the occlusion. The incidence of acute pulmonary oedema was 7 to 8 times as high in patients with

Table V Condition of patients at time of admission

	No. of cases	BP at admission			Pulse rate at
		Normotension	*Arterial hypertension*	*No data*	< 80
Anterior and posterior walls					
Without shock	17	13 (76.5)	2 (11 7%)	2 (11 7%)	1 (5.8)
With shock	1	1	0	0	0
Anterior wall					
Without shock	169	115 (68.1 %)	46 (27.3%)	8 (4.5)	54 (31.9%)
With shock	16	1	0	0	4 (25.0%)
Posterior wall					
Without shock	133	95 (71.5%)	37 (27.9 %)	1 (1.6%)	56 (42.1)
With shock	17	0	0	0	6 (35.3 %)
Without shock in total	319	223 (69.9%)	85 (26.6%)	11 (3.5)	111 (34.8)
With shock in total	34	2 (5.8%)	0	0	10 (29.4)

primarily in individuals suffering from heart diseases. 50 % of the patients discussed here had experienced attacks of angina pectoris only in 34 8 % of all cases had previous heart symptoms never occurred According to the present survey there is no marked difference between patients in whom shock developed and those in whom this complication was absent, except that the incidence of previous infarction was higher in the former than in the latter group

Localization of infarcts

This is based on autopsy findings, if available otherwise the electrocardiographic changes have provided the basis of estimation Data concerning the localization of the infarct were not available in one case and the total material therefore includes only 353 cases (table IV)

The incidence of infarction affecting the anterior wall was found to be higher in the present material than in previous publications

	Anterior wall % (appr)	Posterior wall % (ppr)
Yater et al (38)	48	12
Doscher and Poindexter (9)	54	36
Wright, Marple and Beck (37)	38	33
Hilden et l (19)	40—44	32—36
Present study	52	42

The incidence of fresh lesions affecting the anterior as well as the posterior wall was no higher in patients with shock than in those in whom this complication was absent. Infarction affecting the posterior wall was most common in individuals in whom shock developed but the feature not being identical in the two sexes it can hardly be given any significance Whether the hazards of a development of shock are more or less probable cannot be inferred from an evaluation of the localization of infarcts.

admission			Rhythm at admission			
80—100	> 100	No data	Sinus rhythm	Atrial fibrillation	Ventricular/supra-ventricular tachycardia	No data
9 (52.9%)	7 (41.2%)	0	14 (82.4%)	1 (5.8%)	1 (5.8%)	1 (5.8%)
0	1	0	1	0	0	0
67 (39.6%)	45 (26.6%)	3 (1.9%)	160 (94.7%)	6 (3.5%)	1 (0.6)	2 (1.2)
3 (18.7%)	9 (56.3)	0	13 (81.3%)	0	2 (12.5%)	1 (6.2)
37 (27.8%)	40 (30.1)	0	117 (88.0%)	12 (9.0%)	0	4 (3.0%)
4 (23.6%)	5 (28.8%)	2 (11.7%)	15 (88.3%)	0	0	2 (11.7%)
113 (35.5)	92 (29.2%)	3 (0.9%)	291 (91.2%)	19 (5.9%)	2 (0.6)	7 (2.5%)
7 (20.6)	15 (44.2%)	2 (5.8%)	29 (85.3%)	0	2 (5.8%)	3 (8.8%)

Condition of patients at time of admission

Whether the shock had developed early or late after the occlusion almost all of the patients were in shock when they arrived in hospital at which stage only two of the total number of 34 patients presented normal blood pressure values. This feature should be borne in mind when the grave prognosis is considered. Most of the patients without shock were found to be normotensive (70) whereas blood pressure values above 160/110 were found in about 27 (table V)

According to expectation the pulse rate was higher in patients with shock than in those in whom this complication did not develop although values below 80/min. were recorded in about 29.4 of the cases in the former group. Compensatory tachycardia was a particularly rare finding in patients with shock and infarction affecting the posterior wall, probably because of the higher incidence in this group of atrioventricular blocks. It should be taken into consideration, however whether the reflexogenic compensatory power of the organism, if any is most severely damaged by lesions affecting this site of the myocardium.

On the basis of the initial ECG most of the patients were found to have sinus rhythm. Atrial fibrillation was never recorded in patients with shock and represented rare finding in the other patients (5.9 %) Supraventricular and ventricular tachycardia were seen almost exclusively in patients with shock (5.8 % as against 0.6 %) and always in cases in which the infarction affected the anterior wall.

Condition of patients during hospitalization

It has been mentioned that many of the patients presented symptoms of heart failure and quite often the condition was seen to aggravate in continuation of the infarction. In 20—30 of the cases the symptom developed *after* the occlusion. The incidence of acute pulmonary oedema was 7 to 8 times as high in patients with

Table I Condition of patients at time of admission

	No. of cases	BP at admission			Pulse rate at
		Normotension	Arterial hypertension	No data	< 80
Anterior and posterior walls					
Without shock	17	13 (76.5)	2 (11.7 %)	2 (11.7)	1 (5.8)
With shock	1	1	0	0	0
Anterior wall					
Without shock	169	115 (68.1)	46 (27.3 %)	8 (4.5)	54 (31.9*)
With shock	16	1	0	0	4 (25.0*)
Posterior wall					
Without shock	133	95 (71.5)	37 (27.9)	1 (1.6)	56 (4_.1)
With shock	17	0	0	0	6 (35.3)
Without shock in total	319	223 (69.9*)	85 (26.6)	11 (3.5)	111 (34.8)
With shock in total	34	2 (5.8)	0	0	10 (29.4)

primarily in individuals suffering from heart diseases. 50 of the patients discussed here had experienced attacks of angina pectoris only in 34.8 % of all cases had previous heart symptoms never occurred. According to the present survey there is no marked difference between patients in whom shock developed and those in whom this complication was absent, except that the incidence of previous infarction was higher in the former than in the latter group.

Localization of infarcts

This is based on autopsy findings, if available, otherwise the electrocardiographic changes have provided the basis of estimation. Data concerning the localization of the infarct were not available in one case and the total material therefore includes only 353 cases (table IV).

The incidence of infarction affecting the anterior wall was found to be higher in the present material than in previous publications.

	Anterior wall (appr.)	Posterior wall (appr.)
Y ter et al. (38)	48	L
Dascher and Poindexter (9)	54	36
Wright, Marple and Beck (37)	38	33
Hildén et al. (19)	40—44	32—36
Present study	52	4_

The incidence of fresh lesions affecting the anterior as well as the posterior wall was no higher in patients with shock than in those in whom this complication was absent. Infarction affecting the posterior wall was most common in individuals in whom shock developed, but the feature not being identical in the two sexes it can hardly be given any significance. Whether the hazards of a development of shock are more or less probable cannot be inferred from an evaluation of the localization of infarcts.

Table VII Rate of mortality in 353 patients with infarction

	No of cases	Death occurring within					
		24 hours	1 3 days	3–7 day	7 14 days	14–30 days	30–60 days
Anterior and posterior walls							
Without shock	17	0	2 (11 7%)	2 (11.7)	3 (17.6)	1 (5.8%)	2 (11 7%)
With shock	1	1	0	0	0	0	0
Anterior wall							
Without shock	169	12 (7.1%)	3 (1.8%)	7 (4.1%)	10 (5.8)	19 (11.2%)	2 (1.2%)
With shock	16	2 (12.5%)	2 (12.5%)	4 (25.0%)	1 (6.2%)	0	0
Posterior wall							
Without shock	133	4 (3.0%)	6 (4.5%)	14 (10.5)	9 (6.7)	9 (6.7%)	2 (1 5%)
With shock	17	8 (47 1%)	3 (17.6%)	2 (11.8%)	0	2 (11.8%)	0
Cases in total							
Without shock	319	16 (5.0%)	11 (3.2%)	23 (7.2%)	22 (6.4)	29 (9.1)	6 (1.8%)
With shock	34	11 (32.3%)	5 (14.7%)	6 (17 7)	1 (4.4)	2 (8.8)	0

Table VIII Time of occurrence of shock and course of disease in 34 patients with shock

	No. of cases	Time of occurrence of shock				No specific treatment prior to death	Specific treatment prior death	Restitution to health	
		Within 24 hours	1 3 days	3–7 days	> 1 week			No specific treatment	Specific treatment
Anterior wall	16	10	3	1	2	8	2	4	2
Posterior wall	17	14	0	3	0	11	4	1	1
Anterior and posterior walls	1	1	0	0	0	1	0	0	0
Total	34	25	3	4	2	20	6	5	3

supplies of oxygen morphine preparations digitalis, etc were generous. In all nine of the patients received vasoconstrictor drugs, three of these recovered.

Heart rupture

Rupture of hearts occurred in 10 of the cases in which shock was absent (3 1) i.e. in 9 3 of the fatal cases in this group I the shock group" this feature was seen in one case only (2.9 %) i.e. in 4 of the fatal cases here. The localization of the infarct was of no consequence (affection of the anterior wall 2 9 %, of the posterior wall 3.0)

Only cases are included the diagnosis of which has been verified t autopsy and it is matter of minimal values. But our results and findings by other investigators do not deviat much (W rtman and Hellerstein (35) 4 Yater et al (38) 3 6 McCaine et al. (24) 3.2 % Zinn and Cosby (39) 3 Ekerstrom (11) 3 7 % but are considerably lower than the 13 reported by Dybkær et al. (10)

Table VI Clinical findings and disturbed rhythm during period of admission

	No. of cases	Appearance of heart failure	Aggravation of heart failure	Acute pulmonary oedema	Onset of atrial fibrillation	Onset of A-V block
Anterior and posterior walls						
Without shock	17	7 (41.2)	5 (29.4)	0 (0.0)	1 (5.8*)	(11)
With shock	1	0	0	0	0	0
Anterior wall						
Without shock	169	32 (18.9)	15 (8.8)	11 (6.5)	4 (2.3)	1 (0.6)
With shock	16	6 (37.5)	3 (18.7)	5 (31.3)	0	1 (6.2*)
Posterior wall						
Without shock	133	27 (20.3)	18 (13.5)	(1.5)	5 (3.)	14 (10.5)
With shock	17	5 (29.4)	* (11.8)	5 (29.4)	0	3 (17.6)
Cases in total						
Without shock	319	66 (20.7)	38 (11.9)	13 (4.1)	10 (3.1)	17 (5.3)
With shock	34	11 (32.3)	5 (14.7)	10 (29.4)	0	4 (11.8*)

shock as in those in whom the complication did not develop

Atrial fibrillation occurred in 3 1 % of the patients without accompanying shock whereas this symptom was never encountered in the "shock group

The incidence of atrioventricular blocks (PQ segment above 0.22 sec and atrioventricular blocks of 2nd and 3rd degree) was twice as high in patients with shock as in patients without this complication Nineteen out of a total of 21 atrioventricular blocks were found in cases in which the lesion affected the posterior wall in accordance with the fact that the atrioventricular node is supplied essentially with blood from the right coronary artery (16 20) (table VI)

Prognosis

Possibilities of survival are apparent from tables VII and VIII

30 9 % of the patients in whom shock did not develop died within the first month subsequently few deaths occurred The total percentage of fatal cases in this group amounted to 32.7 death occurring within the first two months after onset of the occlusion.

78.1 % of the patients with shock died death in all cases occurring within the first month usually within the first week

In patients without shock the localization of the infarct was found to be without influence on the prognosis. Death within two months occurred in 31 9 % of the cases in which infarction affected the anterior wall and in 33 1 of those in which the posterior wall was affected.

The rate of mortality among patients with shock was found to be highest if the infarction affected the posterior wall (88 2 died within the first month) as opposed to 62 5 if the infarction affected the anterior wall deaths here also occurring within the first month.

Twenty out of a total of 34 patients in whom shock developed had not received vasoconstrictor drugs five patients who received "non-specific treatment recovered Non-specific treatment implies a therapy in which no vasoconstrictor drugs are administered but

of the patients fail to react to this therapy (21)

Treatment of these latter patients will hardly be successful until the problem of the lacking pressor response has been solved. In his study from 1962 Matthes (27) reports some experiments which Messman et al. carried out on dogs and which indicate that a metabolic acidosis may be responsible for the insufficient action of the vasoconstrictor drugs.

Agress et al. (1) were of the opinion that shock might be attributable to reflexes, the upper dorsal posterior sympathetic roots serving as afferent pathways. Consequently three patients with cardiogenic shock who failed to respond to any one of the therapies were subjected to epidural blockade following which a pressor response was obtained. Notwithstanding that the pressure was raised only one of the patients survived even this result was considered a significant improvement, the rate of mortality otherwise being 100

The most original contribution, probably opening therapeutical possibilities in cases also in which vasoconstrictor drugs fail, is due to Kuhn et al. (21) who used dogs with cardiogenic shock. By pumping engine large quantities of blood were pumped from the superior vena cava to the abdominal aorta — but still the aortic pressure failed to intensify to a degree sufficient to raise the coronary perfusion If, however a balloon catheter was inserted via a femoral artery into the abdominal aorta while the aorta below the obstruction simultaneously was supplied from the superior vena cava, the central aortic pressure could be successfully intensified when the balloon was filled and thus the coronary circulation would be improved. Only one side effect was noted, namely the strength in the hind legs became less in 50 of the animals ischaemia of the medullary spine probably being responsible. This latter complication is hardly prone to occur in man because the medulla in humans ends at a higher site. Renal affection was not seen in any of the experimental animals. So far the method has not been introduced into the clinical routine.

If these or similar measures be found adequate, a centralization of the cardiogenic shock therapy will be highly desirable It will imply however a skilled co-operation of the physician the anaesthetist, and the surgeon

Summary

Following a brief review of the pathophysiology in cardiogenic shock a series of clinical data of 354 patients with acute cardiac infarction is elaborated with particular emphasis on the occurrence of shock.

The diagnosis of cardiogenic shock is verified by electrocardiography or by autopsy findings.

Shock in these patients is defined systolic blood pressure values equalling or below 90 mm Hg.

In the two main groups patients with shock (34 patients) and patients without shock (320 patients) the distribution according to sex was found to be identical the ratio of men to women being 2.4 1 and 2.3 1

The incidence of shock was th same in both sexes (men 9 7 % women 9.3 %) shock generally developed less than 24 hours after the occurrence of the occlusion (in 25 out of 34 patients)

Two thirds of the 354 patients had presented cardiac symptoms prior to the cardia infarction concerned. 50 % of the patients were suffering from angina

Sizes of hearts

Studies on the prognosis e g by Rasmussen and Boe (28) have made it evident that enlargement of the heart is unfavourable to the prognosis of patients with myocardial lesions

Sizes of hearts were determined in 275 of the 354 patients either at autopsy or by X ray It is open to discussion however whether the findings from the two groups patients with and without shock are compatible, evaluation in the former group being based mainly on autopsy findings, in the latter on X ray examination of the thorax prior to discharge from hospital of patients.

The findings included in 83 out of 257 patients without shock (32 2) the hearts were found to be normal in 174 cases (67 8 %) the hearts were found to be hypertrophic.

In the group patients with shock normal conditions were noted in two out of 18 patients (11 1 %) hypertrophy being manifest in 16 cases (88 8 %)

In conformity with the demonstrated higher incidence of shock in patients with a history of hypertension the incidence of cardiac hypertrophy was found to be higher also in this group In addition the generally admitted fact that hypertrophic hearts represent a common finding in cases of infarction (2 25 26 34 37) is substantiated by the present study

Therapeutical aspects

The present study has emphasized that any future development of shock cannot be predicted not even on the basis of clinical data prior to or at the time of admission nor can it be foretold on the basis of the localization of the infarction apparently however it is prone to develop primarily in hypertonic patients presenting cardiac hypertrophy and, not infrequently in patients with a previous history of infarction.

Indeed, the high rate of mortality — close on 80 % — is attributable primarily to the fact that shock in most of the cases was manifest already at the time of admission, many of the patients actually being moribund when they arrived in hospital the same applies to the nine patients in whom shock did not develop until more than 24 hours later This state of affairs may probably be modified by efforts on the part of the admitting physicians and the ambulance staffs. A centralization of therapy should also be taken into consideration.

Symptoms of heart failure are most common in patients with shock, 29 4 % being affected by acute pulmonary oedema, thus indicating the requirement for a more generous administration of digitalis preparations with rapid action. The apprehension here for the complications involved seems highly overrated (3 7 17 32)

The administration of intravenous or intra arterial blood or plasma infusions (5 33) or application of a veno-arterial shunt (4) finds no basis in the haemodynamic conditions in as much as the circulating blood volume is not reduced in cardiogenic shock (22)

With the exception of digitalis, and probably of some of the vasoconstrictor drugs, no preparations are available which through a direct stimulating action on the myocardium can be assumed to remedy the fundamental lesion viz the reduced cardiac output and hence the introduction of drugs which tend to intensify the peripheral resistance must be considered most appropriate. Such a measure will reduce considerably the rate of mortality but unfortunately 50—60

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pectoris about 40 % presented symptoms of heart failure and 8.3 % had a previous history of cardiac infarction

In 52 4 % of the cases the infarction affected the anterior wall in 42.5 it affected the posterior wall and in 5 1 % of the cases the Infarction affected the anterior as well as the posterior wall In patients with shock the infarction was generally found to affect the posterior wall but determination of the site of affection provides no basis on which to draw conclusions as to hazards being more or less marked of occurrences of complicating shocks.

Atrial fibrillation was not recorded in the group of patients with shock, and the symptom was rare in the other patients (5 9 %) Supraventricular and ventricular tachycardia were noted almost exclusively in patients with shock (5 8 % as against 0 6 %) and present only if the infarction affected the anterior wall

Symptoms of heart failure following coronary occlusion were encountered in 20—30 % of the patients. The incidence of complicating pulmonary oedema was 7 to 8 times higher of atrio-ventricular block 2 times higher in patients with shock than in those in whom this complication did not occur

The localization of the infarct was found to be immaterial to the prognosis in patients without shock. In patients with shock the rate of mortality was found to be highest if the infarction affected the posterior wall

Heart rupture occurred in 3 1 of the patients without shock as opposed to 2 9 % in patients with shock Localization of infarction and hazards of heart rupture were not interrelated

Hypertrophy of hearts was diagnosed in 67 8 % of the patients without shock in 88 4 of the patients with shock.

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Primary Premenopausal Haemochromatosis in Two Sisters

By

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Primary haemochromatosis is a disturbance of metabolism characterized by accumulation of excessive haemosiderin in various organs, with their subsequent malfunction. The disease has an earlier onset and is 10 to 20 times more common in males than in females (8). The relative rarity and later onset in women has been attributed to loss of blood during menstruation and pregnancy. Most reported cases in females have occurred at the postmenopausal age. If the disease was premenopausal, the patients had been amenorrhoeic for some reason. It seems that only four definite cases of primary haemochromatosis have been described in normally menstruating women (4, 7, 9, 11). The following two cases of haemochromatosis in sisters, both at the premenopausal age, may therefore be of interest. One of the patients is menstruating normally.

Case report

Case 1. The patient is a 46-year-old housewife first seen in 1960 because of postprandial abdominal discomfort.

Submitted for publication July 1, 1963.

Past history revealed normal regular menstrual periods and two normal deliveries at the age of 28 and 30 years. After the second delivery she began to suffer from postprandial epigastric discomfort, fullness and nausea, for which a cholecystectomy was performed three years later. The gallbladder was said to have been inflamed but without stones. The abdominal symptoms continued unchanged and there appeared periods of diarrhoea with foul smelling and mucoid stools. At the age of 38 she began to complain of intermittent dull ache unrelated to meals and situated in the right subcostal space. At that time she was admitted to another hospital, where an increased bromsulphonphthalein retention and elevated serum alkaline phosphatase activity were noted. In intravenous biligraphy there were no visible concrements but the common bile duct seemed to be somewhat dilated. The abdominal symptoms were interpreted as mostly due to an irritable colon and the therapy consisted of sedatives, with some subjective improvement. During the preceding four years she had lost 4 kg in weight and had noticed increasing tanning of her skin, which persisted over the winter. In 1961 she had vaginitis and suffered from lassitude and thirst, drinking 3–4 l of water per day. The menstrual periods occurred regularly and lasted 4 to 6 days. There had been no iron medication or excessive intake of alcoholic beverages.

Physical examination in 1961 revealed a woman of thin build with diffusely brown



Fig 1 Above haematoxylin-eosin × 100 There is greatly increased connective tissue and some proliferating bil ductules. Below Berlin blue × 160 Increased amount of haemosiderin in the hepatic and reticuloendothelial cells.

skin There were many net like teleangiectases on the face and numerous spider naevi on the chest, shoulders and back. The palms showed erythema. The liver extended 3 cm below the costal margin and was rather smooth and slightly tender The spleen was not palpable. There was tenderness along the colon A small node was palpated in the left lobe of the thyroid. The heart was normal in size and auscultation revealed a grade 2 soft systolic ejection murmur over the precordium. B. P was 115/70 mm Hg

Laboratory findings were as follows Hb 13.2 g/100 ml, red cells 4 3 mill./mm leucocytes 8,300/mm³ differential count eosinophils 2 0 % basophils 0 % metamyelocytes 3 0 % polymorphonuclears 48.5 lymphocytes 39 5 monocytes 7 0 ESR 23 mm/h

The urine contained no protein or excessive bile pigments, the sediment was normal, but there was 60 g of glucose in the 24-hour specimen of urine.

The icteric index was 1 3 alkaline phosphatase 2.2 Bodansky units, bromsulphonphthalein retention 6.3 %/30 min., total serum cholesterol 128 mg/100 ml, SGOT 34 units/ml, LDH 132 units/ml serum protein electrophoresis albumin 59.2 % α_1 2.9 % α_2 11 0 % β 11 1 % γ 15.9 % serum total protein 6 4 g/100 ml, prothrombin time 35 sec. (control 34 sec.)

The fasting blood sugar was 283 mg/100 ml. After oral glucose loading with 1 g/kg of body weight the blood glucose concentrations were 165 at 0 min., 250 at 15 min., 305 at 30 min., 345 at 60 min., 333 at 90 min., 258 at 120 min., 207 at 150 min. and 125 at 210 min.

Serum iron concentration was 240.5 γ/100 ml and the serum iron binding capacity 262 γ/100 ml.

Serum sodium was 140 mEq/l, potassium 4 0 mEq/l, calcium 4.9 mEq/l. After an 8-hour intravenous infusion of 25 U S. P units of ACTH the 24-hour urinary 17 ketosteroids increased from the basal 4 6 mg to 9.2 mg and the 17 ketogenic steroids from 12.0 mg to 29.2 mg, while the circulating eosinophils decreased during the infusion from 370 to 74 cells/mm³

The heart was normal radiologically and in the electrocardiogram. A barium meal showed no oesophageal varices or abnormalities in the stomach, and a barium enema revealed a normal colon.

A skin biopsy specimen from the right thigh showed in the upper part of dermis beneath the membrana propria of the sweat glands and perivascularly abundant pigment-containing cells, which stained with potassium ferrocyanide. The finding was considered to be consistent with haemochromatosis.

A transpleural needle *biopsy of the liver* showed (Prof. H Teir) greatly increased connective tissue and proliferating bil ductules. In the hepatic and reticuloendothelial cells and in the connective tissue there were increased amounts of pigment, which stained readily with Berlin blue (fig 1) The liver specimen was typical of haemochromatosis with portal cirrhosis.

In comment, the patient had primary haemochromatosis with moderate diabetes mellitus, increased skin pigmentation, histological cirrhosis of the liver with mild clinical dysfunction but without signs of heart or adrenal affection. A therapy of repeated phlebotomies

at the blood bank was instituted. Diabetes required 24 units of Novo-lente insulin daily During the two years of observation there has been no change in the liver function tests, serum iron or serum iron binding capacity However the liver is no longer palpable, perhaps indicating progressing cirrhotic change. Subjectively the patient is in good spirits and her abdominal symptoms have become less disturbing. She continues to menstruate, although during the past few months there have been irregularities in the interval.

Case 2 The patient is 42-year-old housewife the only sister of the first patient. She was first seen in 1960 because of dull periumbilical pain.

Past history revealed subtotal resection of the thyroid at the age of 20 because of hyperthyroidism. The menstrual periods had been regular and at the age of 19 she had normal delivery At the age of 36 there was intermenstrual bloodstained vaginal discharge and cervical carcinoma of the uterus was diagnosed. A Wertheim operation was carried out, followed by X-ray irradiation.

During the past five years she had had temporary postprandial nausea, epigastric distention and dull epigastric pain without change in bowel habits and without specific dietary irritants. Barium meal and enema had show repeatedly normal stomach and colon. At the same time she had experienced attacks of palpitations lasting up to 30 min often occurring in association with the postprandial symptoms All symptoms responded favourably to sedatives. No iron medication or excess intake of alcoholic beverages had occurred. Because of the haemochromatosis of her sister she was admitted for further examination in January 1962.

Physical examination showed increased pigmentation of the skin around recent scar in the leg The liver and the spleen were not enlarged The heart was normal on palpation and auscultation In other aspects the examination showed normal conditions.

Laboratory findings were as follows Hb 14.0 g 100 ml, red cells 4.5 mill./mm³ leucocytes 10,000 mm³ differential count eosinophils 3 ,, basophils 0 metamyelocytes 1.5 polymorphonuclears 63.5 lymphocytes 23.5 ,, monocytes 6.5 ESR 21 mm/h.

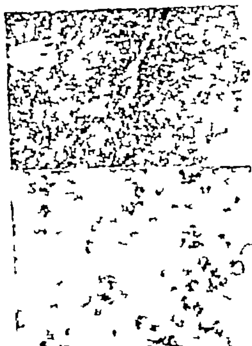


Fig. 2. Above haematoxylin-eosin 100 Normal hepatic structure Below Berlin blue 1,000 Increased amounts of haemosiderin in the Kupffer cells and connective tissue.

The urine showed no protein, glucose or excessive bile pigments and the sediment was normal.

The icteric index was 1 4, alkaline phosphatase 2 6 Bodansky units, bromsulphonphthalein retention 0.3 /30 min., total cholesterol 388 mg/100 ml serum protein electrophoresis albumin 57 2 α_1 5.2 α_2 10 0 β 13 9 γ 13.8 prothrombin time 26 sec. (control 26 sec) thymol turbidity 0.8 Maclagan units.

The fasting blood sugar was 77 mg/100 ml. After an oral glucose loading with 1 g/kg of body weight the blood glucose concentrations were 82 at 0 min 141 at 15 min 167 at 30 min., 110 60 min 98 at 90 min., 75 at 120 min 50 at 150 min. and 70 at 210 min After two-stage oral glucose loading with 50 g at an interval of 30 min. the blood glucose concentration was 86 at 0 min., 148 at 30 min. and 189 at 60 min.

Serum iron concentration was 232 γ/100 ml and the serum iron binding capacity 261 γ/ 100 ml.

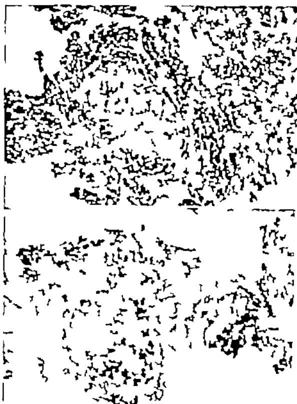


Fig 1 Above haematoxylin-eosin × 100 There is greatly increased connective tissue and some proliferating bile ductules. Below Berlin blue × 160 Increased amount of haemosiderin in the hepatic and reticuloendothelial cells.

skin. There were many net like teleangiectases on the face and numerous spider naevi on the chest, shoulders and back. The palms showed erythema The liver extended 3 cm below the costal margin and was rather smooth and slightly tender The spleen was not palpable. There was tenderness along th colon A small node was palpated in the left lobe of the thyroid. The heart was normal in size and auscultation revealed a grade 2 soft systolic ejection murmur over th precordium. B. P was 115/70 mm Hg

Laboratory findings were as follows Hb 13.2 g/100 ml, red cells 4 3 mill./mm³ leucocytes 8,300/mm³ differential count eosinophils 2.0 %, basophils 0 metamyelocytes 3 0 %, polymorphonuclears 48.5 %, lymphocytes 39 5 %, monocytes 7 0 %, ESR 23 mm/h

The urine contained no protein or excessive bile pigments, the sediment was normal, but there was 60 g of glucose in the 24-hour specimen of urine.

The icteric index was 1 3 alkaline phosphatase 2.2 Bodansky units, bromsulphonphthalein retention 6.3 %/30 min., total serum cholesterol 128 mg/100 ml, SGOT 34 units/ml, LDH 132 units/ml serum protein electrophoresis albumin 59.2 %, α 2.9 %, α_2 11 0 %, β 11 1 %, γ 15.9 % serum total protein 6 4 g/100 ml, prothrombin time 35 sec (control 34 sec.)

The fasting blood sugar was 283 mg/100 ml. After oral glucose loading with 1 g/kg of body weight the blood glucose concentrations were 165 at 0 min., 250 at 15 min., 305 at 30 min., 345 at 60 min., 333 at 90 min., 258 at 120 min., 207 at 150 min. and 125 at 210 min.

Serum iron concentration was 240.5 γ/100 ml and the serum iron binding capacity 262 γ/100 ml.

Serum sodium was 140 mEq/l, potassium 4 0 mEq/l, calcium 4.9 mEq/l. After an 8-hour intravenous infusion of 25 U S. P units of ACTH the 24-hour urinary 17-ketosteroids increased from the basal 4 6 mg to 9.2 mg and th 17 ketogenic steroids from 12.0 mg to 29.2 mg while the circulating eosinophils decreased during the infusion from 370 to 74 cells/mm³

The heart was normal radiologically and in the electrocardiogram. A barium meal showed no oesophageal varices or abnormalities in the stomach, and a barium enema revealed a normal colon.

A skin biopsy specimen from the right thigh showed in the upper part of dermis beneath the membrana propria of the sweat glands and perivascularly abundant pigment-containing cells, which stained with potassium ferrocyanide. Th finding was considered to be consistent with haemochromatosis.

A transpleural needle *biopsy of the liver* showed (Prof H. Teir) greatly increased connective tissue and proliferating bile ductules. In the hepatic and reticuloendothelial cells and in th connective tissue there were increased amounts of pigment, which stained readily with Berlin blue (fig 1) The liver specimen was typical of haemochromatosis with portal cirrhosis.

In comment, the patient had primary haemochromatosis with moderate diabetes mellitus, increased skin pigmentation, histological cirrhosis of the liver with mild clinical dysfunction, but without signs of heart or drenal affection A therapy of repeated phlebotomies

matosis have been elevated in many cases (1 10) Studies of the descendants are therefore certainly indicated. It may well be that in many of the reported sporadic cases of haemochromatosis there may have been undetected subclinical cases among the relatives. However in the family described by us the parents and other known ascendant relatives were probably healthy although not studied more closely The children and brother of our patients have at present serum iron and iron binding capacity values that are within normal limits. Liver biopsy was therefore not considered justified. Admittedly the existence of haemochromatosis in the absence of increased serum iron concentration has been claimed (1) It will be interesting to note whether any of the children will later develop any features of haemochromatosis.

Many of the young amenorrhoeic females with haemochromatosis have had fulminating cardiac disease as the major disturbance (11) Why this should occur predominantly in young amenorrhoeic females is a mystery In the five reported menstruating females, heart disease was not clinically significant.

At autopsy the adrenals have been found to have heavy deposits of haemosiderin and in particular the zona glomerulosa has been fibrotic (3) Despite this the adrenal cortical function is usually normal clinically and as judged by hormonal analyses (8) Diabetes, which may be of the pure insulin lack variety responds usually well to insulin treatment It is usually the liver involvement that forms the chief threat by progressing to portal type pigment cirrhosis. For reasons not known the development of hepatic cell carcinoma is more frequent in cirrhosis due to haemochromatosis than in the usual Laënnec type.

Summary

Haemochromatosis in two sisters is reported. One of them menstruates normally and appears to be the fifth certain case of haemochromatosis in a menstruating woman. The other patient underwent hysterectomy six years prior to the diagnosis. The children and brother of the patients appear to be healthy as judged by serum iron concentrations and iron binding capacities. The current concepts of the pathogenesis, mode of inheritance and main clinical features of haemochromatosis are briefly discussed

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Serum sodium was 141 mEq/l potassium 4.3 mEq/l and calcium 5 0 mEq/l. After an 8-hour intravenous infusion of 25 U S P units of ACTH the 24-hour urinary 17 ketosteroids rose from the basal 9.2 mg to 21 9 mg and the 17 ketogenic steroids from 17 6 mg to 93.3 mg

The heart was normal in the X rays and ECG

A transpleural needle *biopsy of the liver* revealed (Prof H Teir) a fairly normal hepatic structure with no signs of cirrhotic change Increased amounts of haemosiderin were seen in the Kupffer cells and connective tissue and to a smaller extent in the hepatic cells. There also was some increase in the bile pigment (fig 2)

In comment the patient had a subclinical haemochromatosis without apparent dysfunction of the liver heart or adrenals. The abnormal result in the two-stage oral glucose loading test, however pointed to a disturbance in insulin secretion For treatment the patient was, like her sister made a blood donor

Familial studies

The family history revealed no consanguinous marriages and no liver disease, brown skin or diabetes in the known relatives. The mother had died probably from disseminated tuberculosis at the age of 48 and the father had succumbed to a probable cardiac infarct at the age of 43 The only brother is in good health, with serum iron of 141 γ/100 ml. The 16- and 18-year-old daughters of the first patient are clinically healthy with serum iron concentrations of 133 5 and 132 γ/100 ml and serum iron binding capacities of 325 and 327 γ/100 ml respectively The 24-year-old daughter of the second patient has serum iron of 157.5/100 ml and serum iron binding capacity of 319/100 ml

Discussion

The basic metabolic defect in primary haemochromatosis is as yet unknown The concept of an inhibitive action of intestinal mucosal ferritin on iron absorption has been largely abandoned (8) However it is generally assumed that in haemochromatosis the absorption of iron from normal diets is above normal and that this leads to an increased saturation of the plasma transferrin and an increase in the tissue apoferritin and haemosiderin, which in turn leads to secondary functional and structural changes in the affected organs The view of MacDonald and Mallory (5 6) of haemochromatosis as a variant of alcoholic or nutritional cirrhosis seems not to have received support.

The normal iron excretion in the faeces and urine is less than 1 mg daily (2) In fertile females menstruation causes an average additional loss of 1 mg daily In haemochromatosis an excess of 20 g of iron has been estimated to exist at the time of diagnosis (8) If the development of clinical disease takes 20—30 years, it means a positive iron balance of 2 to 4 mg daily (8) Thus normal menstrual blood loss is a rather effective preventive factor in predisposed women. The five normally menstruating cases of haemochromatosis presumably therefore represent a variant of the disease with more eager iron absorption Unfortunately accurate methods for measuring iron absorption rate are still lacking

The mode of inheritance of haemochromatosis is generally thought to have an autosomal recessive pattern (8) In an extensive liver biopsy study of 48 relatives of 16 known haemochromatosis patients, 32 had an at least electronmicroscopically increased iron content in the liver and the results were interpreted to show an intermediate form of inheritance, in which the heterozygous subject has some abnormality but to a lesser degree than the homozygous (12) Some severe cases with early onset in siblings have been reported in consanguinous marriages (8) Serum iron values of the, at that time, healthy children of patients with haemochro-

matosis have been elevated in many cases (1 10) Studies of the descendants are therefore certainly indicated It may well be that in many of the reported sporadic cases of haemochromatosis there may have been undetected subclinical cases among the relatives. However in the family described by us the parents and other known ascendant relatives were probably *healthy although not studied* more closely The children and brother of our patients have at present serum iron and iron binding capacity values that are within normal limits. Liver biopsy was therefore not considered justified. Admittedly the existence of haemochromatosis in the absence of increased serum iron concentration has been claimed (1) It will be interesting to note whether ny of the children will later develop any features of haemochromatosis.

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Eosinophilia in Peripheral Blood and Inflammatory Exudate in Non-specific Proctocolitis

By

Povl Riis and Poul Anthonisen

Since Andresen (1) first suggested allergy as pathogenetic possibility in non-specific proctocolitis evidence has accumulated in favour of the view that at least some cases of this disease have an allergic pathogenesis. Thus several authors have presented series of proctocolitis patients with various allergies (2, 12, 18, 21 23 27) high titres of antibodies against milk proteins have been found in some patients with proctocolitis (11 24) and antibodies against human colon mucosa and other pathological globulins ha been demonstrated in the serum of patients with the disease (7 14 23).

The finding in patients with proctocolitis of eosinophilia in the peripheral blood or in the local inflammatory process would further support the conception that hypersensitivity mechanisms are involved in non-specific proctocolitis. Our material has, therefore, been analysed in these tw respects.

Submitted for publication June 28, 1963.

Material and methods

The material comprises 74 unselected patients with proctocolitis. All clinical phases of the disease are represented, from the most active to completely quiescent. Most of the patients have been followed for several months, many for almost two years.

Blood eosinophil counts were mad in 67 of the patients. Blood eosinophilia has been recorded if at any time the counts have been above 400 μl.

For the evaluation of eosinophilia in the inflammatory exudate replica method was employed (3) during sigmoidoscopy a unit consisting of an ordinary cork, onto the broad end of which a protein-coated circular glass disc is glued (fig 1) is inserted through the sigmoidoscope with the help of a tampon holder until the glass disc lightly touches the mucosa. After about one second contact the unit is withdrawn and the replica stained like blood smear.

With this method it has been demonstrated (4) that secretions from normal proctosigmoidal mucosa and from the mucosa of irritable colon patients contain only mucus strands, bacteria, and occasional epithelial cells, but no or – in rare cases – negligible number of

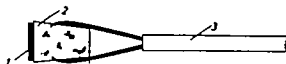


Fig 1 Replica sampling unit fastened in tampon holder 1 glass disc, 2 cork, 3 tampon holder

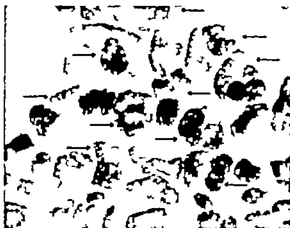


Fig 2 Pronounced eosinophilia in inflammatory exudat from proctocolitis patient. Arrows indicate eosinophils (appr × 1 000)

inflammatory cells. In contrast replicas from patients with active proctocolitis are characterized by numerous leucocytes, mainly neutrophilic and eosinophilic granulocytes, but also lymphocytes, and plasma cells. Admixture of erythrocytes is a frequent finding Fig 2 shows a typical example with pronounced eosinophilia.

Local eosinophilia has been recorded if the relative number of eosinophils in the replicas on one or more occasions has clearly exceeded that found in inflammatory exudates of known non-allergic nature

Results

Table I shows the results. Blood eosinophilia was an occasional finding in the present material (28 %) while eosinophilia in the inflammatory exudate was found in the great majority of cases (80 %) Both these percentages are minimum figures as a few of the patients without eosinophilia in the blood or in the inflammatory exudate were receiving systemic corticosteroid therapy

Table I Eosinophilia in peripheral blood and inflammatory exudate in present material of proctocolitis patients

	Eosinophilia		No. of pat. examined
	Present	Absent	
Peripheral blood	19 (28%)	48	67
Inflammatory exudate	59 (80%)	15	74

4 patients in systemic corticosteroid treatment included.

3 patients in systemic corticosteroid treatment included.

In patients with blood eosinophilia, the degree of the eosinophilia was often inconstant. It was not influenced by topical steroid treatment but was promptly eliminated when systemic steroid therapy was instituted

The degree of eosinophilia in the inflammatory exudate often showed fluctuations from one examination to another In some patients, however this local eosinophilia was constantly pronounced in spite of marked changes in the activity of the disease. Thus a large relative number of eosinophils has been observed in the inflammatory exudates of practically symptom free patients with macroscopically normal proctosigmoidal mucosa and a small — although pathological — total number of inflammatory cells in the replicas In sharp contrast are the patients in whom eosinophilia was never demonstrated in the inflammatory exudates. Topical or systemic steroid therapy usually reduced the total number of leucocytes (and erythrocytes) in the replicas. The relative number of eosinophils was as a rule unaffected by topical treatment but was reduced in some cases

Table II. Data from the literature concerning eosinophilia in the local inflammatory process of proctocolitis

	Authors	No. of examinations (patients)	Eosinophils
Biopsies	Lumb & Protheroe (15)	180 (150)	Extensive infiltration in third of cases
	Truelove & Richards (26)	111 (42)	Often numerous
	Dick & Grayson (9)	62 (62)	Not mentioned
	Mann (19)	126 (126)	Not commonly present
Operation and/ or autopsy specimens	Goldgraber et al. (10)	159 (124)	Tissue eosinophilia in 40% of cases
	Lumb (16)	152 (152)	Present
	Valdes-Dapena & Vilardell (28)	30 (30)	No eosinophilia
Mucosal secretion	Cain et al. (8)	?	Often numerous
	Bercovitz (5)	?	Quite constantly demonstrated
	Boddington & Truelove (6)	?	Often numerous
	Present material	(74)	Eosinophilia in 80% of cases

by systemic steroid therapy It is however remarkable that pronounced local eosinophilia persisted in a few patients during intensive steroid therapy topical as well as systemic.

Two patients — one with and one without local eosinophilia — had large numbers of basophilic granulocytes in their inflammatory exudates.

Discussion

The literature contains but little information concerning the number of eosinophilic granulocytes in the blood of patients with non-specific proctocolitis. Machella and Hollan (17) made blood eosinophil counts in three patients with active proctocolitis before during and after systemic cortisone therapy in only one of these was significant blood eosinophilia present before the institution of treatment. Kirsner and Palmer (13) observed fall in the number of blood eosinophils in most of the cases of proctocolitis treated with cortisone, but their paper does not state blood eosinophil counts numerically Rasager (22) found that 12 patients with active proctocolitis had higher blood eosinophil counts than 12 patients in full clinical remission (on an average 320 as compared to 127 eosinophils/μl) Nine of the patients with active disease had counts above the author upper normal limit of 200/μl. Three patients were examined both during remission and during acute exacerbation. In all the acute attack was preceded and accompanied by a rise in the number of blood eosinophils.

In the present material that comprises all degrees of proctocolitis activity 28 per cent of the patients had at least on one occasion blood eosinophil counts above our upper normal limit of 400/μl.

Concerning eosinophilia in the local inflammatory process of proctocolitis the literature discloses great disagreement (table II) In mucosal biopsies some authors have not noted eosinophils (9 19) while others have demonstrated

distinct eosinophilia in several cases (15 26) Lumb and Protheroe (15) saw pronounced eosinophilia in a third of their cases. In operation specimens Valdes-Dapena and Vilardell (28) found no eosinophilia, while Lumb (16) did see eosinophils in his series although he did not state the degree of infiltration In operation and autopsy specimens Goldgraber et al (10) found tissue eosinophilia in 40 per cent of cases Some workers have devised methods to obtain proctosigmoidal mucosal secretion for microscopical examination (5 6 8) They have all been impressed by the often very great numbers of eosinophils in preparations from patients with proctocolitis, but the exact frequency of eosinophilia in the inflammatory exudate does not appear from these publications

Local eosinophilia was not an occasional finding in the present material it was found in the great majority of our patients with proctocolitis which suggests that eosinophilic granulocytes play an important part in the inflammatory process of this disease In other inflammatory colon diseases that we have had the opportunity to examine (e g infectious enteritis and diverticulitis) no such local eosinophilia has been observed (4)

In mucosal biopsies from proctocolitis patients McAuley and Sommers (20) found mast cells more frequently than in biopsies from patients with other colon diseases. In the present material only two patients had local basophilia — one concomitant with eosinophilia — but special staining methods were not employed

Summary

The finding in patients with proctocolitis of eosinophilia in the blood or in the local inflammatory process would support the hypothesis that hypersensitivity mechanisms are involved in non-specific proctocolitis

The present material comprises 74 patients and represents all degrees of disease activity

Eosinophilia in the inflammatory exudate was evaluated using the authors replica method for cytological examination of the proctosigmoidal mucosal secretion Distinct local eosinophilia was found in 80 per cent of the cases. In only two patients was basophilia demonstrated but special staining methods were not employed

Blood eosinophil counts were made in 67 of the patients. Twenty-eight per cent of these had counts above the upper normal limit of 400 per microlitre.

The finding of distinct eosinophilia in the inflammatory exudate of most patients with non-specific proctocolitis suggests that eosinophilic granulocytes play an important part in the inflammatory process of this disease.

Acknowledgement

Aided by grants from Kong Christian X's fond Alfred Benzon fond" and P Carl Petersen fond

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Collagen Disease Associated with Intestinal Malabsorption and Sprue-like Changes in the Intestinal Mucosa

By

S. Toivonen, E. Pitkänen and M. Siurala

The involvement of the gastrointestinal tract is not uncommon in collagen diseases. This is in particular true of scleroderma, which in addition to the esophagus commonly involves the small intestine (1, 2, 5, 7–9, 11, 13–15). Various gastrointestinal disturbances have also been described in systemic lupus erythematosus, dermatomyositis and polyarteritis nodosa (3, 10). Conclusive data on the anatomical and functional state of the small intestine in the latter conditions are lacking, however.

The present report deals with two patients with collagen disease. Gastrointestinal studies disclosed in both cases intestinal malabsorption and sprue-like changes in the mucosa of the small intestine. This study is a part of a larger project to study the state of the gastrointestinal tract in various collagen diseases.

Case reports

Case 1. A 51-year-old laborer had suffered during the past three years from swelling and tenderness of skeletal muscles and high fever and lately from abdominal pain and diarrhea. The symptoms appeared in bouts lasting up to several months. Later he also had dyspnea and chest pain during exertion. At the age of 49 years coronary infarction was suspected.

In the active phases of the disease a highly elevated sedimentation rate of the erythrocytes, leucocytosis with marked eosinophilia and high sustained level of serum glutamic-oxalacetic transaminase were observed.

On admission he was slightly dyspnoeic and cyanotic, and the skeletal muscles were tender and firm in consistency. The blood pressure was normal and there was moderate tachycardia. No marked signs of congestive heart failure could be demonstrated. The blood circulation time was lengthened and the heart was enlarged (1,390 cm³/m²). No heart murmurs were heard.

The ECG showed sinus rhythm and a lengthened PQ-interval (0.28), the RS complex was 0.16 and RT interval 0.36. The QRS complex was somewhat distorted. A tracing taken two years previously had been normal.

There was moderate anemia and leucocytosis. Eosinophil count was 2,066/mm³, serum iron 4 µg %, TIBC 1,250 µg %, serum potassium 3–6 mEq/l, and phosphorus 2.5 mg %. Electrophoretic analysis revealed diffuse hypergammaglobulinemia, and cryoglobulin was demonstrable in serum.

Submitted for publication July 2, 1963.

n the diaphragm and the skeletal muscles, particularly in limbs.

Microscopy of skeletal muscles (fig. 2), (Prof. Derek Denny Brown, Department of Neurology Harvard University Medical School, and Prof. Harald Teir Department of Pathology University of Helsinki) The skeletal muscles showed zones of relatively normal muscle surrounding fairly discrete zones of recent severe destruction with abortive regeneration but very scanty evidence of reconstruction of muscle fibers. There was marked inflammatory response in the damaged areas. I some areas the blood vessels showed cellular infiltration in addition to swelling and homogenization. The larger vessels were normal and the condition did not have the appearance of periarteritis nodosa.

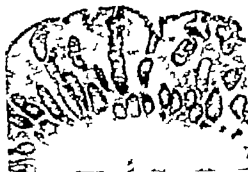


Fig 3. Case 2. Section f biopsy specimen from the mucosa of the distal duodenum. No villi are seen. Severe epithelial alterations are present Moderate inflammatory cell infiltration is seen throughout the lamina propria. Muscularis mucosa ppears intact. H-E. × 90.

Comments

A 31 year-old male suffered from bouts of muscle pain fever eosinophilia and diarrhea. The blood creatine phosphokinase level as well as the level of other muscle enzymes was markedly elevated. The bsorption of itamin B_{12}, iron, calcium, fat, itamin A and d-xylose was impaired. The mucosa of the small intestine showed trophy of the villi Muscle pecimens obtained before and after death revealed generalized muscle disease, presumably ascular in basis.

Ca Female 54 ears, was admitted with history of high fever join swelling, bdominal pain, diarrhea and anemia On admission the fever was 38 40 C The abdomen was diffusel tender The knees, ankles and the wrist joints were stiff and ender

There was macrocy ic and megaloblastic anemia Hemoglobin was 8.90 g erythrocytes ere 2.37 mill mm³ leucocytes 6,600/mm and MCH was 38 The serum iron was 20 μg Serum alcium, potassium and phosphorus levels were normal. ESR was 100 mm h Electrophoretic analysis revealed hypergammaglobulinemia of 33...

The examinations of liver kidneys, heart and lungs rev aled nothing of interest.

The Waaler Rose reaction, antistreptolysin test, Wassermann and Coombs reactions, LE cell test and LE titer were repeatedly negative. The latex fixation test, however was positive.

The serum levels of glutamic-oxalacetic transaminase, lactic dehydrogenase, creatine phosphokinase, aldolase and alkaline phosphatase were normal.

Cutaneous and muscle biopsies revealed normal muscle tissue and increased basophilic degeneration and destruction of the cutaneous collagen fibers.

Gastrointestinal studies The fecal fat content was 70 of the dry substance. V tamin A load resulted in onl slight increase in the serum vitamin A level (8—43 μ 100 ml) The d-xylose excretion was 6 g in 5 hours. Schilling test I was 0 and Schilling test II (with intrinsic factor) was 1 5 of the given dose.

Roentgenologic examination of the whol gastrointestinal tract showed deficiency pattern in the upper small intestine A small intestinal biopsy exhibited regressiv changes similar to those encountered in case 1 (fig 3)

Comments

A 54-year-old female with signs suggestive of some collagen disease had malabsorption of vitamin B_{12}, iron fat and d-xylose nd severe alterations in the intestinal mucosa.



Fig 1 Case 1 Section of a biopsy specimen from the mucosa of th distal duodenum Atrophy of the mucosa is apparent. V ll are absent. Severe alterations are present in the epithelial cells. There is moderate inflammatory cell infiltration. Muscularis mucosa is almost normal H E. × 90



Fig 2 Case 1 Biopsy from a skeletal muscle. Severe degenerative and inflammatory changes are present. The muscle fibers are swollen and have lost their constrictions. In some reas the muscle fibers are replaced by granulation tissue Swelling and homogenization f the wall of the small arteries is sible H. an Gieson × 190

Waaler Rose, AST LE factor and WaR tests were normal. Kahn and citolipin tests were positive in serum but not in cerebrospinal fluid The Kolmer and treponema pallidum immobilization tests ere positive in serum. The Kahn test was already positive 94 years previously when the patient was under treatment for lymphogranuloma e nereum (type inguinal-aden tis) Although no history of primary luetic infection could be obtained, the patient had been treated with series of bismuthum injections and later with massive doses of intramuscular penicillin.

Serum lactic acid concentration was 15–20 mg serum creatinine 0 84 and creatine 1 48 mg The urinary creatine excretion was 300 mg daily The o-toluidine test for hemoglobin or myoglobin was positive in urine. There was no increase in the number of red cells or leucocytes in urine. Serum glutamic-oxalacetic transaminase level was markedly elevated up to 220 units. Lactic dehydrogenase activity was 1,500 units. An electrophoretic analysis on starch gel revealed elevation of the fast moving component of lactic dehydrogenase (muscle lactic dehydrogenase) The fructose diphosphate aldolase was 80 units and creatine phosphokinase 380 units. The activities of serum leucyl aminopeptidase and glucose-6-phosphate dehydrogenase were normal.

Gastrointestinal studies Roentgen examination of the stomach and the small intestine revealed nothing of interest. The vitamin A tolerance test was pathological (fasting value was 14 μ/100 ml 4 hours after the vitamin A load the value was 23 μ/100 ml) The d-xylose excretion was 1.5 g/5 hours. Urinary radioactivity after the ordinary Schilling test was 5 5 of the dose administered. Serum iron was 4 μ/100 ml and serum calcium 8.9 mg/100 ml of serum.

Peroral biopsy from the distal duodenum (Rubin et al multipurpose biopsy tube) revealed changes similar to those present in the primary malabsorption syndrome (fig 1). There were no villi. The epithelial cells showed marked alterations. The striated border was discernible only in some places. The cell borders were indistinct. The nuclei varied in form and size. There was moderate inflammatory-cell infiltration in the lamina propria The muscularis mucosae was almost normal. Periodic acid stain revealed nothing suggestive of Whipple s disease

Course of disease The patient received antibiotics and corticosteroids, but without benefit. Three eeks after admission the patient died suddenly

Autopsy findings Macroscopically the heart muscle was diffusely ery fibrotic, the color of the myocardium was whitish and it was dense in consistency The endocardium and the coronary arteries were ntact In the myocardium there were no signs of old or recent infarction The same fibrotic changes were found

the basis of their own cases and of those reported by others that in many patients the signs of malabsorption have preceded the development of reticulosis. However in the present cases with collagen disease signs other than intestinal dominated the clinical picture, and in case 1 they had preceded the onset of the gastrointestinal symptoms. In seven patients with primary malabsorption studied so far no signs of collagen disease have been encountered in any of the cases. There were no signs of skeletal muscle involvement, nor did the serum enzymes reveal any abnormality. These findings are in agreement with those of Scudamore et al. (12) who found no signs of systemic involvement of mesenchymal organs in any of 272 patients with intestinal malabsorption.

As a further possibility it should be noted that the systemic signs and the sprue-like syndromes might be manifestations of different hereditary defects, a possibility which at present cannot be neglected nor proved.

Summary

Two patients with undetermined collagen disease are described. In both there were distinct signs of intestinal malabsorption, and peroral small-intestinal biopsy disclosed changes characteristic of non-tropical sprue. In the patient with generalizated muscle disease there was a striking increase in the blood level of various muscle enzymes, in particular of creatine phosphokinase.

Acknowledgements

The authors wish to express their thanks to Prof. Derek Denny-Brown, M. D., Department of Neurology Harvard University Medical School, and to Prof. Harald Teir M. D. II Department of Pathology University of Helsinki, for histological examinations of the biopsy specimens and for valuable suggestions.

Aided by grant from the Sigrid Jusélius Foundation.

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Discussion

The clinical picture of the patient with a generalized muscle disease (case 1) was characterized by acute exacerbations with muscle pain fever leucocytosis, eosinophilia and diarrhea. The clinical picture as a whole showed many signs common to the collagen diseases. The periodic acid stain revealed nothing suggestive of Whipple's disease. In spite of positive syphilitic reactions the adequate antiluetic therapy given in connection with the primary infection the clinical course and the anatomical findings were inconsistent with an active syphilitic process.

The signs of general skeletal muscle involvement clearly dominated the clinical and pathoanatomical picture of the disease. Creatine phosphokinase a specific muscle enzyme showed a more than 300-fold increase in the serum activity The serum values of muscle lactic dehydrogenase aldolase and glutamic oxalacetic transaminase were constantly at a very high level. The presence of myoglobinuria was indicated by the positive o-toluidine test in the absence of hematuria and of signs of hemolytic state.

The morphologic findings (fig 2) in the skeletal muscles revealed many signs common to dermatomyositis or to polymyositis as described by Denny Brown (4) On the other hand the nature of the morphological changes and the presence of a marked vascular lesion suggested that the muscular alterations might have been caused by some generalized disease of the smaller blood vessels. The blood vessel changes, however did not have the appearance of polyarteritis nodosa. Nor were the changes consistent with lupus erythematosus. Hence, it was not possible to classify this rare type of collagen disease.

The functional impairment of the small intestine was demonstrated by defective absorption of calcium, fat, vitamin A, d xylose, iron and of vitamin B_{12}. A per oral biopsy of the small intestine exhibited features similar to those encountered in the primary malabsorption syndrome (fig 1)

In the second patient the case history suggested some kind of collagen disease. However repeated examinations (LE cells LE titer) failed to establish a diagnosis of systemic lupus erythematosus. The findings were also not compatible with dermatomyositis or scleroderma. In this patient too the gastrointestinal studies showed changes as in non-tropical sprue. The patient was encountered during screening examinations for intestinal malabsorption in patients with collagen disease Seven recently observed consecutive patients with collagen disease have been studied so far In addition to the patient described above, two further patients revealed disturbed absorption of vitamin A and of d xylose. However intestinal biopsy showed normal intestinal mucosa in both

The explanation for the coexistence of collagen disease and malabsorption may be that the blood vessels, the muscularis, the submucosa and the lamina propria of the intestinal mucosa may participate in the systemic mesenchymal reaction and lead to functional disability and anatomical changes of the small bowel. However no marked changes were found in the muscles or blood vessels of the small intestine in the present cases.

The possibility that the intestinal changes were primary should also be considered This possibility has recently been suggested in connection with another form of systemic disease, the so-called reticuloses. Gough et al. (6) claimed on

Acta Medica Scandinavica. Vol. 175, fasc. 1, 1964

From the Wihuri Research Institute (Head Pentti I. Halonen, M.D.) Helsinki, Finland

Precordial Isotope Dilution Curve in Aortic Valvular Disease

By

JUHA HAKKILA and KARI A. PIETILÄ

Dye or isotope dilution curves have been used in the study of hemodynamics in valvular disease, using peripheral recording in the effort to interpret the type of the valvular disease and to determine the cardiac output in cases of stenosis. In aortic insufficiency retrograde dye dilution examinations have also been made for estimation of the regurgitation amount (15). If mitral or tricuspid valvular disease is concomitant with the aortic valvular disease, diagnosis of the different valvular diseases and evaluation of their significance become more difficult (4, 6).

The precordially recorded isotope dilution curve has in recent years gained use in the study of hemodynamics in heart disease (8, 10, 11, 12, 13, 14) as it gives more direct evidence of circulation in the heart and lungs. Precordial isotope dilution determinations have been performed on patients with mitral valvular disease (10, 11) and some observation has been published on the use of the method in aortic valvular disease (11, 14).

The purpose of the present is to study the precordial isotope curve in pure aortic valvular disease and in aortic valvular disease combined with other valvular lesions. The results obtained from the curve are correlated with the clinical impression of the severity of the disease and to the roentgenological findings in thoracic X-ray.

Methods and material

For the double-peaked precordial isotope dilution determination 30–40 μC of I^{131}-albumin was injected into the cubital vein with the patient supine. The curve was recorded by a scintillator having a cylindrical lead collimator with a half value angle of 30 degrees focussed on the pulmonary artery and left atrium. The method has been described in an earlier paper (5). From the curve the cardiac index was calculated, the peak-to-peak time indicative of the pulmonary circulation time, the pulmonary blood volume based on the latter and the left-right peak ratio, which means the relationship of the extrapolated areas of the left and right heart peaks (fig. 1).

The series consisted of 45 patients whose diagnosis was in most cases based on the typical picture. There were 24 males and 21 women. The mean age was 43 years (range 17–67).

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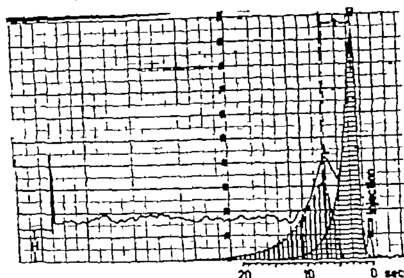


Fig. 1 Precordial isotope dilution curv from normal subject. R = right and L = left heart peak, - extrapolated portion of the curve, - curve drawn by subtraction for the left side of heart, ☰ = area of the right heart and ||| = left heart peak, H = height of the equilibrium level and t peak-to-peak time

Values from the curve Cardiac index (CI) 5.5 l/min., peak-to-peak time (t) 4.8 sec pulmonary blood volume (PVB) 440 ml/m

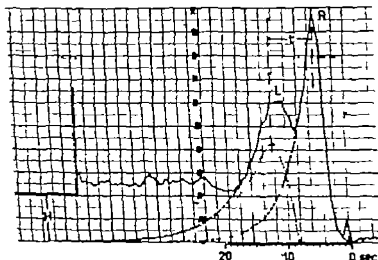


Fig 2. Precordial curv from patient with mild aortic stenosis. The shape similar to the normal curve. Results from the curv (symbols as in fig 1) CI 3.8 l/min., 6.8 sec., PBV 430 ml/m

peak time was the longer the higher the pressure in the left atrium measured t operation. The curves (fig 6) did not greatly differ in shape from those earlier obtained from patients with mitral stenosis.

Of these patients, 17 had "pure" calcified aortic stenosis, one had congenital valvular stenosis, and one subvalvular stenosis due to septal hypertrophy Pure aortic insufficiency was present in 15 patients and 6 patients had combined stenosis and insufficiency Five patients had aortic valvular disease combined with mitral stenosis. Three patients needed mitral commissurotomy one of these an aortic valvulotomy in addition. In one case there was tricuspid mitral and aortic valvular stenosis (verified at autopsy)

The patients were distributed according to their symptoms and physical performance into two groups, following the criteria of the New York Heart Association Mild cases with no symptoms or in which the symptoms, such as stenocardia, syncope and dyspnea, did not restrict activity (classes I and II) and severe cases in which the above mentioned symptoms greatly restricted activity or were present already at rest (classes III and IV)

The control series consisted of 30 subjects with no evidence of cardiopulmonary disease, mean age 41 years (range 18—64)

From the conventional postero-anterior and lateral chest films the relative heart volume per sq.m (7) was calculated and attention was paid especially to dilatation of the left ventricle which was regarded as probable when the long axis of the heart was lengthened, the retrocardiac space narrowed, the posterior wall of the left ventricle overlapped the margin of the inferior vena cava, and there were no signs of enlargement of the right ventricle.

The correlations of the values calculated from the precordial curves were determined in relation to each other and to the relative heart volume. The statistical significance of the differences between the means for the groups and the normal series was calculated.

Results

Shape of dilution curve

In mild cases of aortic stenosis the dilution curve differed only slightly from the curve for a normal subject (figs. 1 and 2) In severe cases of stenosis the left heart peak was usually slightly higher than normal, while the right heart peak was unchanged In the most severe cases exhibiting signs heart failure the right heart peak was broad and the peak to-peak time prolonged (fig 3)

The curves of patients with aortic insufficiency generally differed from the normal The left heart peak was remarkably high frequently higher than the right heart peak, and it had a sharp rise and fall (fig 4) This shape of curve was seen in all cases with roentgenologically enlarged left ventricle but no signs of left ventricle insufficiency A curve of normal shape was seen in the two mildest cases in which the heart was roentgenologically within normal limits. In patients with heart failure the curve was similar in shape to that in patients with aortic stenosis in heart failure. On the whole, in most cases in which the peak to-peak time clearly exceeded the upper limit of 9 sec seen in normal subjects, the chest films showed signs of pulmonary congestion or septal lines.

Patients with combined stenosis-insufficiency in the aortic valve gave curves that in 4 cases were similar to those in aortic insufficiency and in 2 cases to those in aortic stenosis In all these cases there was dilatation of the left ventricle in the chest films.

The values obtained from the precordial curves and the relative heart volume of normal subjects and patients with aortic valvular disease are shown in fig 5 The mean values are given in table I

In the cases in which the aortic valvular disease was combined with mitral stenosis the peak to-peak time was increasingly prolonged with the severity of mitral stenosis. A normal peak to-peak time was seen in only one patient, who had normal PCV and PA pressures in heart catheterization and the area of the mitral valve was calculated to be 2 6 cm The peak to-

Fig 1 Precordial isotope dilution curve from normal subject. R = right and L = left heart peak, = extrapolated portion of the curve = curve drawn by subtraction for the left side of heart, ☰ = area of the right heart and |||= left heart peak, H = height of the equilibrium level and = peak-to-peak time.

Values from the curve Cardiac index (CI) 5.5 1/min., peak-to-peak time ( ) 4.8 sec., pulmonary blood volume (PVB) 440 ml/m

Fig 2 Precordial curve from patient with mild aortic stenosis. The shape similar to the normal case Results from the curve (symbols as in fig 1) CI 3.8 1/min 6.8 sec., PBV 430 ml/m

peak time was the longer the higher the pressure in the left atrium measured at operation. The curves (fig 6) did not greatly differ in shape from those earlier obtained from patients with mitral stenosis.

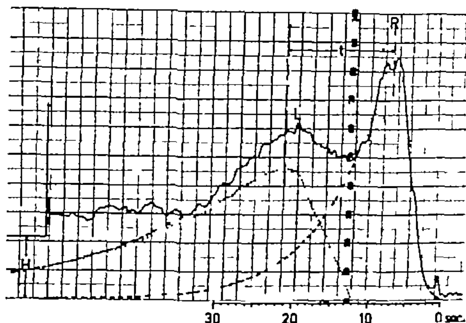


Fig 3 Precordial curve from patient with aortic stenosis in heart failure. Right heart peak prolonged. Results from the curve (symbols as in fig 1) CI 1.9 l/min., t 13.9 sec., PBV 450 ml/m

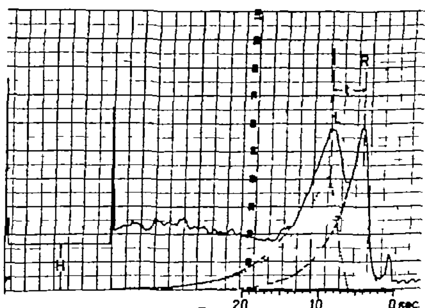


Fig 4 Precordial curve from a patient with aortic insufficiency Prominent left heart peak. Results from the curve (symbols as in fig 1) CI 4 7 l/min t 4.5 sec., PBV 350 ml/m

Correlation study

Aortic stenosis Compared with normal persons there was a highly significant drop in the cardiac index ($p = 0.001$) The decrease was clearly evident already in the group of mild cases and there was no difference between the mild and the severe cases.

The peak to-peak time was prolonged already in patients with mild aortic stenosis as compared with normal subjects ($p = 0.01$) The difference between the

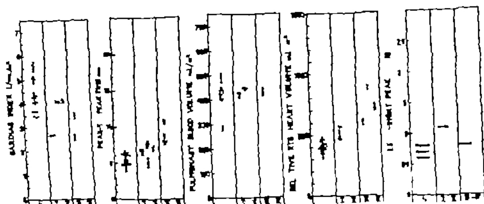


Fig. 5. Comparison of results for normal subjects and patients with aortic valvular disease grouped into mild (classes I—II) and severe cases (classes III—IV, N.Y. Heart Ass.) Symbols: N = normal subjects, = aortic stenosis, ○ = aortic insufficiency, Δ = combined aortic stenosis and insufficiency (cf. table I)

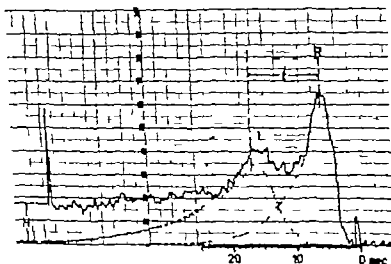


Fig. 6. Precordial curve from patient with mitral and aortic stenosis. Peak-to-peak time prolonged. Results from the curve (symbols as in fig. 1): CI 3.2 l/min, 10.9 sec, PBV 390 ml m

whole group and the normal group was highly significant (p < 0.001).

The pulmonary blood volume per sq.m did not significantly differ in normal subject and patients with aortic stenosis (p =)

The left right peak ratio was increased from the normal only in the group of severe cases (p = 0.05).

Aortic insufficiency. The cardiac index values in this group revealed no statistically significant differences from the val

Table I Results obtained by the precordial dilution method and thorax X-ray in 30 normal subjects and 40 patients with aortic valvular disease Comparison of the means. The patients grouped into mild (classes I—II) and severe (classes III—IV N Y Heart Ass.) cases (cf fig 5)

Diagnosis	Cardiac index (ml)	Peak to-peak time (sec)	Pulmonary blood vol (ml)	Relative heart (ml)	Left-right peak ratio
Normal subjects	4 40	6.0	423	403	0.69
Aortic stenosis					
Mild	3 18	7.3	387	544	0.86
Severe	2.84	9.7	438	678	0.93
Aortic insufficiency					
Mild	4.10	6.7	427	5.4	1.62
Severe	3.46	9.9	488	732	1.54
Aortic stenosis and insufficiency					
Mild	4.35	6.2	448	574	0.93
Severe	2.90	7.8	367	625	1.53

ues in normal subjects. Owing to valvular regurgitation it is possible that this method of determination of cardiac out put does not give correct values

Compared with the normal group the peak to-peak time of the aortic insufficiency group was significantly prolonged (p = 0 001) This difference was not clearly evident in mild cases.

The pulmonary blood volume did not differ from that in the normal group (p =)

The left right peak ratio was significantly increased from the normal both in the mild cases and the whole group (p = 0 001)

Both the cases of aortic stenosis and of aortic insufficiency showed a negative correlation between the cardiac index and the peak to-peak time (p = 0 05 and 0 001) In the cases of aortic stenosis there was additionally a significant positive correlation between the peak to-peak time and the left right ratio (p = 0 01) A similar correlation was not seen in aortic insufficiency which on the contrary had a high left-right ratio despite, in some cases a normal pulmonary circulation

The patients with aortic stenosis had a larger average relative heart volume than normal subjects (p = 0 001) No significant difference was present between mild and severe cases (p =) The subjects with aortic insufficiency were found to have an increased relative heart volume as compared with normal subjects (p = 0 001) The average for mild cases revealed no statistical difference.

Discussion

In cases of aortic valvular disease the cardiac output value determined by Fick s method has been found to be normal at rest and decreased only in the most severe cases of aortic stenosis (2) It would seem possible that in cases of aortic insufficiency regurgitation from the aorta back to the heart would lead to erroneous values in the precordical meas-

urements. It might be assumed that the retarded passage of radioactivity from the left ventricle would increase the area of the curve obtained from the primary circulation, thus decreasing the cardiac output value. However the values obtained, which correspond to those obtained for normal subjects under similar conditions, are evidence of the serviceability of this method also in cases of aortic insufficiency

With the exception of very mild cases with a roentgenologically normal heart, patients with aortic insufficiency generally had in their curve a prominent second peak that differed from the curve for normal subjects and patients with aortic stenosis. It is evident that with the method used larger proportion than usual of the radioactivity is within the range of measurement of the scintillator secondary to the enlarged left ventricle and regurgitation.

I severe cases of aortic stenosis or aortic insufficiency the pulmonary circulation time was prolonged. Some of these cases were in heart failure and interstitial pulmonary edema and pulmonary congestion were observed in chest films. The changes in the pulmonary circulation time determined by the precordial technique ar in good agreement with the observ tions that the diastolic pressure in the left ventricle remains normal in aortic stenosis and aortic insufficiency until there is failure of the left ventricle 16, 19 With elevation of the diastolic pressure in the left ventricle the pressure in the left atrium also increases and congestion of the pulmonary veins occurs. The normal pulmonary circulation time is herefore evidence gainst the presence of insufficiency of the left ventricle in cases with aortic lvula disease. In cases of aortic insufficiency in heart failure the second prominent peak was not obtained probably because of slow pulmonary circulation.

A large proportion of aortic valvular diseases are a combination of aortic and mitral valvular diseases (1,3) In these cases t is necessary especially if surgery is under consideration, to determine the relative degree of the two valvular lesions. In most cases of combined aortic mitral valvular stenosis the cardiac output is lowered, and the pressure gradient between the aorta and the left ventricle is low in relation to the degree of valvular stenosis (6) The precordial isotope curve may be useful in these cases not only in determining the cardiac output but also in assessing the degree of severity of the mitral valvular stenosis. In these cases the pulmonary circulation time, which in compensated aortic stenosis or aortic insufficiency is within the normal range, appears to be prolonged according to the degree of severity of the mitral valvular stenosis, similarly to the retardation earlier observed in cases of pure mitral stenosis.

It has been reported that the heart size of patients with aortic stenosis as seen n chest films in the frontal projection is not correlated to the severity of the valvular stenosis (9) Evidence in th same direction is the bservation made in the present study that no significant difference existed between the relative heart volume in mild and severe cases. However compared w th normal subjects the volume was increased. On the other hand, in aortı valvula insufficiency the heart size has been observed to be correlated to the degree of val ular lesion (9) which seems to be indicated also by the finding in the present work of difference in the relative heart size in mild and severe cases.

Summary

A precordial isotope dilution examination was performed on 45 patients with aortic valvular stenosis or insufficiency a part of whom had a combined aortic valvular disease or additional mitral stenosis. The controls were 30 normal subjects.

From the two-peaked dilution curve were calculated the cardiac output pulmonary circulation time and pulmonary blood volume (based on the peak to-peak time) and the ratio of areas of the peaks The results and the relative heart volume in the different types of valvular disease, distribution according to the severity of the condition are presented

The pulmonary circulation time was normal in the group with aortic stenosis and in that with aortic insufficiency with the exception of severe cases with signs of failure of the left side of the heart.

Patients with aortic stenosis exhibited a larger than normal second peak of the dilution curve. In aortic insufficiency this peak was high and sharp The concomitant presence of a hemodynamically significant mitral stenosis was seen in a prolonged pulmonary circulation time.

The correlation of the results was subjected to statistical treatment.

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Gastric Lesion in Vitamin B_{12} Deficiency of Fish Tapeworm Carriers

By

M. Siurala, I P Palva and W Nyberg

Tapeworm pernicious anemia is a vitamin B_{12} deficiency state caused by *diphyllobothrium latum* This parasite competes with its host for the dietary B_{12} vailable in the intestine (2, 8) presumably by splitting th intrinsic factor B_{12} complex (7 9 10) The released vitamin is then taken up by the worm (8, 9 10) All degrees of vitamin B_{12} deficiency are seen in carriers of the fish tapeworm (2 4 11) It is possible that the sever lesion of the intrinsic factor secreting area of the stomach commonly found in tapeworm pernicious anemia (15) may predispose to vitamin B_{12} depletion (4 6 21 for further references see 6 21) There is,however,some evidence to suggest that the functional and morphological lterations in the gastric mucosa may in some patients be caused by the deficiency state 6, 15 16) Accordingly a study of the condition of the gastric mucosa in tapeworm carriers before and after expulsion of the worm may give valuable information on the gastric response to various degrees of vitamin B_{12} deficiency

Submitted for publication July 2 1963.

Material and methods

Seventy-five patients, 42 males and 33 f males, with tapeworm infestation were studied. Their mean age was 44.5 years.

The following examinations were perfor med

Peripheral blood count and bone marrow aspiration.

Biopsy from various parts of the gastric mucosa using Sielaff suction biopsy tube (Richard Wolf, GmbH, Western Germany) Two hundred and twenty specimens, or about 3 specimens per patient, were taken t the first examination, and 120 specimens from 40 patients at the re-examinations. N complications occurred during the procedure. The specimens were fixed with 5 % neutral formalin and stained by the hematoxylin-eosin, hematoxylin-van Gieson and PAS methods.

Histalog test meal (7.5 mg/kg) Specimens were withdraw 30, 60 and 90 min. after injection.

Uropepsin excretion (21) 12 hours (over night) excretion.

Schilling test (14) using 0.6 µg of ^{60}Co B_{12} (Abbot Lab North Chicago, Ill.)

Vitamin B_{12} activity in serum using *Euglena gracilis*, strain Z (13)

I patients with epigastric distress a roentgen examination of the stomach was per

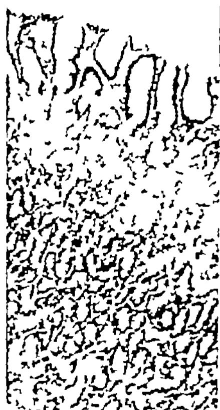


Fig 1 Male 46 yrs. Hg 12.2, RBC 4 46, bone marrow normal. Schilling I test 20.2 serum B_{12} 216 pg/ml Histalog test meal peal secretion 62 mEq/L Uropepsin excretion 22 UP units/h. Histologically normal gastric mucosa (H E, × 100)



Fig 2. Male, 57 yrs. Hg 14.2 RBC 4 11 bone marrow giant metamyelocytes. Schilling I test 0.8 Serum B_{12} 22 pg/ml. Achlorhydria. Uropepsin excretion 4 0 UP units/h. Histological findings superficial gastritis with some loss of normal body glands (H E, × 100) Five months after expulsion of the worm, blood count, bone marrow Schilling test, and serum B_{12} were normal. The histological changes in the gastric mucosa were unchanged.

formed In no case could gastric ulcer or gastric cancer be detected

A follow-up examination was performed approximately 6 months after expulsion of the worm Forty patients were re-examined.

Results

A Before expulsion of the worm

1 *Morphological and functional state of gastric mucosa*

The morphological condition of the gastric mucosa as shown by the biopsy specimens of the 75 subjects was as follows

Normal gastric mucosa in 28 patients (37 %)

Superficial gastritis (inflammatory cells beneath the surface or throughout the mucosa without loss of body glands) in 19 patients (25 %)

Slight atrophic gastritis (slight loss of normal body glands) in 14 patients (19)

Moderate atrophic gastritis (considerable loss of body glands, figs. 3 and 6) in 8 patients (11)

Severe atrophic gastritis (complete or almost complete loss of body glands, figs. 4 and 5) in 6 patients (8 %)

Thus the incidence of gastritis was 63 %


Fig. 3. Female, 19 yrs. Hg 12.2, RBC 3.78. Bone marrow normal. Schilling I test 6.2 %. Achlorhydria. Uropepsin excretion 1.0 UP units/h. Histological findings: severe atrophic gastritis with intestinal and pseudopyloric metaplasia and marked inflammatory signs (H-E, 100)


Fig. 4. Male, 64 yrs. Hg 12.0 RBC 2.94, bone marrow megaloblastic. Schilling I test 7.0 %, serum B_{12} 84 pg/ml. Achlorhydria. Uropepsin excretion 1.0 UP unit/h. Complete intestinalization of the gastric mucosa with almost absent inflammatory reaction (H-E, 100)

I testinal metaplasia (intestinal type of glands in the gastric body mucosa figs 4 and 5) was found in 14 patients, and pseudopyloric metaplasia (antral type of glands, figs. 3 and 6) in 19 patients. Severe inflammatory signs were noted in in 11 cases. Large numbers of eosinophils were present n the biopsy specimens of 14 patients.

Hydrochloric acid (HCl) secretion was tested in 71 patients Achlorhydria was found in 26 cases 37) low peak secretion of 1—50 mEq l) in 20 28 normal secretion (52—100 mEq l) in 20 (28) and high secretion n 5 cases (7) The incidence of chlorhydria and low HCl secretion is rather high (65 %)

The correlation of the HCl secretion with the morphological state of the gastric mucosa is shown in fig. 8. It appears that the correlation with the histological findings is very good

Uropepsin (UP) excretion was estimated in 53 patients. Low values were present n 21 cases (40 %) normal values (11—50 UP units/h) in 27 cases (51) and high values n 5 cases 9 %)

A satisfactory correlation was obtained between th UP excretion and the morphological stat of the mucosa. Thus in patients with a normal mucosa the



Fig 5 Same patient as in fig 4 5 months after expulsion of the worm. Hg 13 6 RBC 4 64 Schilling I test 6 8 %. Achlorhydria. Uropepsin excretion 2 0 UP units/h. Still complete intestinalization of the gastric mucosa (H E, × 100) Twelve months later the Schilling I test was 4 5



Fig 6. Female, 46 yrs. Hg 7 8, RBC 2.01 bone marrow megaloblastic. Schilling I test 0.5 %, serum B_1 10 pg/ml. Achlorhydria. Uropepsin excretion 3 0 UP units/h. Histologically severe atrophic gastritis with pseudopyloric metaplasia (H E, × 100)

mean UP excretion was 31 1 in those with superficial gastritis 24 0 in those with slight and moderate atrophic gastritis 12 5 and in patients with severe atrophic gastritis 1 8 UP units/hr

2 Hematological findings

Fourteen patients suffered from megaloblastic anemia In 2 patients without anemia giant metamyelocytes in large numbers were found in the bone marrow smears In addition the serum B_1 level of all these 16 patients was also low and hence this group of patients was considered to have a *manifest vitamin B_{12} deficiency* The remaining 59 patients were without clinical signs of vitamin B_{12} deficiency although 28 of them had a decreased serum B_{12} level and/or abnormal Schilling test value.

In the group with *manifest B deficiency* all but one had gastritis (severe atrophic gastritis 5 moderate or slight atrophic gastritis 8 superficial gastritis 2) Eighty one per cent of them had achlorhydria, and their mean UP excretion was as low as 8 1 UP units/h On the other hand in the group *without manifest B_1 deficiency* only 15 of 59 patients (25) had atrophic gastritis. Achlorhydria was present in 22 % of the cases, and the mean UP excretion was 25 6 UP units/h.

3 Schilling test

An ordinary Shilling test was performed in 73 patients. The results (fig 9) were

Normal test value (> 10 % excretion) in 30 cases (41)

Subnormal test value (5—10 % excretion) in 11 cases (15 %)

Low test value (< 5 % excretion) in 32 cases (44 %)

The test values were low in 15 and subnormal in one of the 16 patients with manifest B_{12} deficiency

The results of the Schilling test are correlated with the morphological state of the gastric mucosa in fig. 9 All patients with severe atrophic gastritis and most patients with a less severe atrophic gastritis had pathological test values. It was also found that a higher incidence of low Schilling test values existed among patients with intestinal metaplasia. Further a correlation between the disturbance of the B_{12} absorption and the quality and quantity of the inflammatory cell infiltration was also observed.

The average peak HCl secretion was 26 3 mEq l in patients with low Schilling test values, 29 4 mEq/l in those with subnormal, and 33.8 mEq/l in those with normal values. The mean values for UP excretion in the corresponding groups were 15 8, 20 9 and 27 0 UP units/h.

4 Serum vitamin B assay

Serum B_{12} level was assayed in 51 patients The following results were obtained

Normal value (> 150 pg ml) in 14 patients 2

Subnormal value 100—150 pg ml) in 8 patients 16

Low value 100 pg ml) in 29 patients 57)

The serum B_{12} was low in all patients with manifest B_{12} deficiency



Fig 7 Same patient as in fig 6, 9 months after expulsion of the worm. Hg 12.1 RBC 4.10, Schilling I test 26 0 Free hydrochloric acid present. Uropepsin excretion 3 UP units/h. Histologically the body gland layer appears almost normal, with signs of superficial and deep inflammation (H E, 100)

The occurrence and degree of gastritis is compared with the serum level of B_{12} in table I It is seen that the incidence and the severity of gastritis increases with decreasing B_{12} levels. A tedency to decreasing B_{12} values was also noted in patients with intestinal metaplasia.

Thurteen of 29 patients with low serum B_{12} level and 2 of 14 with normal B_{12} level had achlorhydria. The mean peak secretion values in these groups were 31 and 42 mEq l respectively

The mean uropepsin excretion in patients with low serum B_{12} levels was 20 7 and in those with normal values 24 0 UP units h



Fig 5 Same patient as in fig 4 5 months after expulsion of the worm. Hg 13 6, RBC 4 64 Schilling I test 6 8 Achlorhydria. Uropepsin excretion 2.0 UP units/h. Still complete intestinalization of the gastric mucosa (H E, × 100) Twelve months later the Schilling I test was 4.5



Fig 6. Female, 46 yrs. Hg 7.8, RBC 2.01 bone marrow megaloblastic. Schilling I test 0.5 %, serum B_{12} 10 pg/ml. Achlorhydria Uropepsin excretion 3.0 UP units/h. Histologically severe atrophic gastritis with pseudopyloric metaplasia (H E, × 100)

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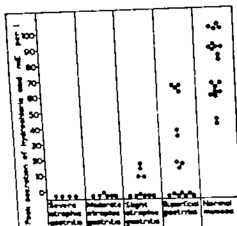


Fig 8 Comparison of the results of Histalog test meals with the histological findings.

● Tapeworm carriers with hematological signs of B_{12} deficiency

○ Tapeworm carriers without hematological signs of B_{12} deficiency

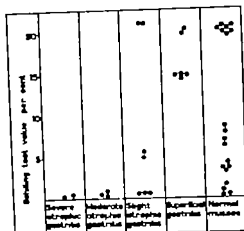


Fig 9 Comparison of the Schilling test values with the histological findings. Symbols as in fig 8.

clinical signs of vitamin B_{12} depletion. In general, however the regenerative changes observed were not impressive. Thus, complete return to normal of a severely damaged gastric mucosa was not observed in any case

The secretion of HCl was examined before and after expulsion of the worm in 31 cases Of 15 patients with achlorhydria 5 revealed free HCl after worm cure. A significant increase in the HCl secretion was seen in 2, and a decrease in 2 persons out of 11 with originally low HCl values. In 3 cases the HCl secretion was normal, and remained so after expulsion of the worm

The changes in uropepsin excretion revealed no consistent pattern.

Comments on follow-up examinations

After expulsion of the worm some improvement in the mucosal state was observed in 8 of the 40 re-examined patients. The signs of remission were however not distinct. Achlorhydria subsided in 5 cases. Thus, on the whole the functional and morphological alterations of the gastric mucosa remained unchanged in spite of apparently successful treatment with correction of the signs of B_{12} deficiency

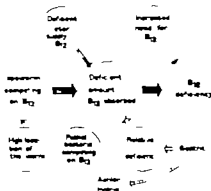


Fig 10 Pathogenetic interrelationships of various factors involved in the development of the tapeworm pernicious anemia.

Table I Degree of gastritis compared with the serum level of vitamin B_{12}

	Serum level of B_{12} (pg/ml)		Total
	0−100	> 100	
Normal mucosa	8	12	20
Superficial gastritis	7	5	12
Atrophic gastritis	15	4	19
Total	30	21	51

Table II Change of the gastritis at follow-up examination

	Remission of gastritis	No change	Progression of gastritis	Total
B_{12} deficiency with hematological signs	3	5	—	8
Low Schilling test and low serum B_{12} without hematological signs	2	3	—	5
No sign of B_{12} malabsorption	3	14	2	19
Total	8	22	2	32

Comments on results obtained before expulsion of the worm

On account of the described results it seems justifiable to state that a correlation exists between an increasing vitamin B_{12} deficiency and progressive atrophic changes in the gastric mucosa In spite of some parallelism found between decreased HCl secretion and uropepsin excretion respectively and signs of a disturbed B_{12} absorption the correlation between these functions was not quite distinct.

In order to evaluate the suggested causal relationship between the state of the gastric mucosa and the B_{12} deficiency the patients were re-examined after adequate treatment (expulsion of the worm and B_{12} injections) The results are given below

B Follow up examinations

Fourty patients were seen approximately 6 months after expulsion of the worm. After that a second and a third re-examination was performed on 8 and 2 patients, respectively

In all but 3 cases the signs of B_{12} deficiency had subsided at the time of re-examination. In one patient the serum B_{12} level was still low in spite of a normal Schilling test. In two others the serum B_{12} level had become normal but Schilling test values remained low (figs. 4 and 5) One of these three patients had a megaloblastic anemia at the first examination. All three patients had complete atrophy of the body mucosa with achlorhydria and low uropepsin excretion at all examinations (figs. 4 and 5)

The response of the histological changes in the gastric mucosa to treatment is shown in table II Eight patients whose mucosa was normal before and after expulsion of the worm are not included in the table. It appears that some remission in the state of the gastric mucosa occurred in 8 patients. Five of these had distinct

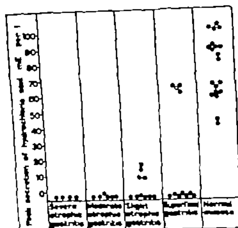


Fig. 8. Comparison of the results of Histalog test meals with the histological findings.

● Tapeworm carriers with hematological signs of B_{12} deficiency

Tapeworm carriers without hematological signs of B_{12} deficiency

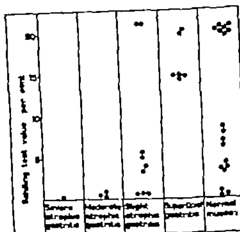


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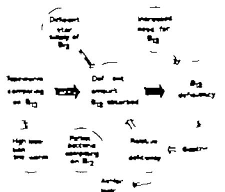


Fig. 10. Pathogenetic interrelationships of various factors involved in the development of the tapeworm pernicious anemia.

The signs of remission were, however, not distinct. Achlorhydria subsided in 3 cases. Thus, on the whole, the functional and morphological alterations of the gastric mucosa remained unchanged in spite of apparently successful treatment with correction of the signs of B_{12} deficiency.

Discussion

The results suggest a causal relationship between the disturbed B_{12} absorption and the gastric mucosal lesions found in fish tapeworm carriers. This is in agreement with earlier results obtained by Siurala (15, 16) who found a high incidence of superficial-atrophic gastritis in patients with tapeworm anemia.

It should be noted, however, that the mean age of the patients was so high that the expected incidence of gastritis must have been considerable. In addition, gastritis, although mainly of a less severe degree, was also found in many patients with no signs of B_{12} depletion. Hence, it is likely that the occurrence of gastritis in a number of the studied cases was merely coincidental. In the remaining cases, where a causal relationship is thought to exist, we have the possibilities that the gastritis had either predisposed to the deficiency, or that it had been caused by the deficiency state.

If gastritic changes were caused by the deficiency state they might well have subsided after expulsion of the worm and after the B_{12} doses received with the Schilling tests. No real improvement of the mucosal changes could be established, however. On the other hand this result by no means excludes the possibility that the mucosal changes nevertheless are initiated or aggravated by the B_{12} deficiency state, as there is some evidence to suggest that a gastritis once developed tends to remain or progress and very rarely heals (19). In this connection attention may be drawn to other cell damage seen in B_{12} deficiency, e. g. to atrophic changes found in the mucosa of the oral cavity.

All patients with severe atrophic gastritis had a disturbed B_{12} absorption, which suggests that the mucosal lesion may be one of the predisposing factors for the development of B_{12} deficiency in tapeworm carriers. It may be mentioned that Siurala et al (17, 18) studying patients without clinical signs of pernicious anemia and without tapeworm infestation, commonly found defective absorption of radiovitamin B_{12} and low serum B_{12} level in patients with complete atrophic gastritis. This finding was later confirmed by Glass et al. (3). It is remarkable, however, that a less severe mucosal lesion seemed not to be able to interfere with the absorption of radiovitamin B_{12} in the first mentioned studies.

It is obvious that no uniform explanation can be given for the development of the gastric changes in tapeworm carriers. In many cases the gastritis is probably purely coincidental. In some patients, mainly in those with a severe mucosal lesion, gastritis may have predisposed to development of B_{12} deficiency. In some cases again — in particular in those with a less severe degree of gastritis — it is possible that the mucosal changes are caused or aggravated by the deficiency state.

Gastritis is not by any means the sole factor predisposing to B_{12} deficiency in connection with fish tapeworm infestation. An increased need for, or decreased supply of, vitamin B_{12} may be additional factors that may account for the development of the deficiency in some tapeworm carriers (2, 11, 12). The period of infestation, the location (1) and the size and metabolic activity of the tapeworm are also factors which may be of great importance (2). The achlorhydria — commonly found in the present series — might as well as the tapeworm itself predispose to growth of a pathological bacterial flora in the upper intestine, which may further decrease the amount of B_{12} avail-

able for the host (20). The various pathogenic factors possibly involved in the development of vitamin B_{12} deficiency in tapeworm carriers are schematically presented in fig. 10.

Summary

The anatomical and functional state of the gastric mucosa in B_{12} deficiency was studied in *75 carriers of the fish tapeworm*. Fourteen of the patients suffered from megaloblastic anemia, two had giant metamyelocytes in the bone marrow, 28 revealed a decreased absorption of radiovitamin B_{12} without hematological changes, and the remaining 31 patients had no signs of disturbed B_{12} absorption.

The *gastric suction biopsy* (220 specimens from 75 patients) revealed normal mucosa in 28, superficial gastritis in 19, slight atrophic gastritis in 14, moderate atrophic gastritis in 8, and severe atrophic gastritis in 6 patients.

The *secretion of hydrochloric acid* (Histalog test, 71 patients) was absent in 26, low (peak secretion of 1–50 mEq/l) in 20 and normal in 25 patients. The results correlated well with the histological findings.

Uropepsin excretion was low in 21, normal (10—50 UP units/h) in 27, and high in 5 cases. The results correlated satisfactorily with the histological findings.

Schilling test values (73 cases) were low in 32, low normal (5—10 %) in 11 and normal in 30 patients. There was a marked increase in the severity and incidence of gastritis, and decrease in HCl secretion with decreasing absorption of vitamin B_{12}.

Serum level of vitamin B_{12} (*Euglena gracilis*, 51 cases) was low in 29, low normal (100–150 pg/ml) in 8, and normal of gastritis revealed an apparent increase with decreasing level of vitamin B_{12}. With respect to *HCl secretion* and *uropepsin excretion*, the correlation was not satisfactory.

The patients with manifest B_{12} deficiency (16 cases) had all but one gastritis, and most (13) achlorhydria. All 6 patients with severe atrophic gastritis had abnormal Schilling test values and low serum B_{12}.

Follow-up examinations were performed on 40 patients approximately 6 months after successful expulsion of the worm. In 8 cases the histological changes of the gastric body mucosa showed some remission. The regenerative changes observed, were, however, not impressive. An increase in HCl secretion was noted in 7 cases.

Acknowledgment

The study was aided by a grant from the Sigrid Jusélius foundation.

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Cutis Verticis Gyrata and Mental Deficiency in Sweden

I. Epidemiologic and Clinical Aspects

By

Hans Olof Akesson

Cutis verticis gyrata is an unusual con formation of the scalp characterized by the presence of folds and furrows that impart to the scalp a corrugated and, in some instances, a gyrate appearance (82)

Though most of the hundreds of cases of cutis verticis gyrata described have been in people of normal intelligence, people of subnormal intelligence seem to be far more prone to the disorder Nevertheless, w know only little about cutis erticis gyrata in mental deficiency for almost the only literature on this combination consists of reports on one or two cases. Thus not much is known about its prevalence incidence geographical distribution age of onset, mortality association w th other physical abnormalities, sociomedical consequences, hereditary background and causation. For this reason, in 1961 and 1962 I made a study of ll the cases of cutis erticis gyrata among the inmates of Swedish institutions for the mentally deficient.

I shall report the results of this study in tw parts. The present report deals with the epidemiologic and clinical aspects of these cases, with some of the genetic aspects and with the preliminary laboratory data. Later on I shall report the results of a familial analysis.

Survey of literature

Cutis verticis gyrata in persons of normal intelligence

So little having been written about cutis verticis gyrata together with subnormal intelligence, I shall begin with a review of th literature on cutis erticis gyrata in persons of normal intelligence.

Cutis verticis gyrata is not disease in itself it is only one manifestation of larger morbid process, which may be of several different kinds.

Many uthors hav tried to divide cutis verticis gyrata into primary forms of idiopathic nature, and secondary forms caused by other abnormal processes. But until we know much more bout cause and effect in this disorder there will always be cases in which it is impossible to say whether the scalp disorder is primary or secondary

The disease in which cutis erticis gyrata most commonly occurs in persons of normal intelligence is pachydermoperiostosis, or idiopathic hypertrophic osteoarthropathy (42, 86, 95, 102) This disease occurs almost only in

Submitted for publication July 18, 1963

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Acta Medica Scandinavica. Vol. 175, fasc. 1 1964

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Cutis Verticis Gyrata and Mental Deficiency in Sweden

I. Epidemiologic and Clinical Aspects

By

Hans Olof Åkesson

Cutis verticis gyrata is an unusual conformation of the scalp characterized by the presence of folds and furrows that impart to the scalp a corrugated and, in some instances, a gyrate appearance (82).

Though most of the hundreds of cases of cutis verticis gyrata described have been in people of normal intelligence, people of subnormal intelligence seem to be far more prone to the disorder. Nevertheless, we know only little about cutis verticis gyrata in mental deficiency, for almost the only literature on this combination consists of reports on one or two cases. Thus not much is known about its prevalence, incidence, geographical distribution, age of onset, mortality, association with other physical abnormalities, sociomedical consequences, hereditary background and causation. For this reason, in 1961 and 1962 I made a study of all the cases of cutis verticis gyrata among the inmates of Swedish institutions for the mentally deficient.

I shall report the results of this study in two parts. The present report deals with the epidemiologic and clinical aspects of these cases, with some of the genetic aspects and with the preliminary laboratory data. Later on I shall report the results of a familial analysis.

Survey of literature

Cutis verticis gyrata in persons of normal intelligence

So little having been written about cutis verticis gyrata together with subnormal intelligence, I shall begin with a review of the literature on cutis verticis gyrata in persons of normal intelligence.

Cutis verticis gyrata is not a disease in itself; it is only one manifestation of a larger morbid process, which may be of several different kinds.

Many authors have tried to divide cutis verticis gyrata into primary forms of idiopathic nature, and secondary forms caused by other abnormal processes. But until we know much more about cause and effect in this disorder, there will always be cases in which it is impossible to say whether the scalp disorder is primary or secondary.

The disease in which cutis verticis gyrata most commonly occurs in persons of normal intelligence is pachydermoperiostosis, or idiopathic hypertrophic osteoarthropathy (42, 86, 93, 102). This disease occurs almost only in

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men and nearly always begins during or after puberty. In its complete form it is characterized as follows: the fingers and toes grow broader, the hands and feet grow larger and spade-shaped, and the arms and legs develop into thick cylinders; the features grow coarser and the skin on the face becomes hypertrophic and thrown into folds, giving the patient an anxious expression; cutis verticis gyrata develops on the scalp; roentgenograms show periosteal ossification in the limbs, mostly along the distal surfaces of the long bones, less in the phalanges of the metacarpal and metatarsal bones; no changes are seen in the shape of the sella turcica. In incomplete forms, one or more of these characteristics may be missing, including the cutis verticis gyrata. Pachydermoperiostosis is caused by hereditary mechanisms, but the manner of transmission is still obscure; as a rule, it occurs only among the siblings of affected persons, but occasionally their parents are also affected. It sometimes develops during advanced pulmonary disease, particularly malignant pulmonary tumors. Altogether about a hundred cases of pachydermoperiostosis have been described; exhaustive surveys of the disease have been given by several authors, including Schilling et al. (91), Tornblom et al. (103) and Vogl and Goldfischer (111). It was first reported in the mid-nineteenth century, and there was much dispute about its origin among the famous clinicians of the time, including Friedreich, Erb, Virchow, Sternberg and Marie. It was originally thought to be a form of acromegaly, but in the end it was recognized to be a disease in itself.

In view of the likeness between pachydermoperiostosis and acromegaly, it is interesting that cutis verticis gyrata has also been found in acromegaly in cases in which the diagnosis of acromegaly has been confirmed both clinically and histologically (1, 32, 40, 89, 93, 100, 117). In some of the old reports on this combination, however, the abnormalities described are more like those in pachydermoperiostosis than in acromegaly (19, 62, 71, 72).

Cutis verticis gyrata may be caused by nevi in the scalp (18, 21, 22, 25, 39, 49, 60, 64, 74, 75). Then it is generally present at birth. Another way in which this form differs from the others is that it is sometimes found in females (29, 34, 44, 61, 66). Furthermore, it is generally well demarcated from the surrounding scalp and raised above it, which is usually not so otherwise. The folds also seem to be made up of nervous tissue, and to be closely related to neurofibroma (44, 59, 67). (Note that cutis verticis gyrata has been found together with neurofibromatosis (107).) Because of these differences, it has been suggested that this form of corrugation in the scalp be called cerebriform nevus rather than cutis verticis gyrata.

Cutis verticis gyrata may also develop during different types of infection (10, 16, 56, 76, 79, 84, 99, 109), wounds (7, 12, 46, 68, 98) and eczema (68, 83, 101). There are occasional reports of it being brought on by psoriasis (108), leukemia (78), folliculitis keloidalis (15, 87), acanthosis nigricans (70) and pemphigus (96).

Some authors have contended that it is sometimes caused by the scalp growing faster than the skull; they adduce in support its frequent association with brachycephaly and microcephaly (2, 5, 108). We still know too little about the disorder, however, to be able to say whether it can be brought on by mechanical forces alone.

It has been observed in several persons with schizophrenia (30, 35, 105). Most of these persons have had the catatonic form of schizophrenia.

In spite of all the foregoing examples, most people of normal intelligence with cutis verticis gyrata do not have any other physical or neurologic disorder (see ref.). In this great majority of cases, probably of several different origins, the disorder nearly always develops after puberty, and nearly always occurs in males. As a rule these patients seem to be the only members of their families affected; only occasional instances of familial occurrence are recorded.

To summarize this survey on cutis verticis gyrata in persons of normal intelligence: It may develop in several different diseases but usually it is the only sign of abnormality present. As a rule, it is impossible to explain how it has come about.

Cutis verticis gyrata in persons of subnormal intelligence

McDowall (78) seems to have been the first to draw attention to a combination of cutis verticis gyrata and mental deficiency, in 1893

he reported having observed this scalp disorder in two microcephalic male idiots, one of whom also suffered from epilepsy and hemiparesis. The same year Cowan (23) reported tw more cases of this combination one patient was a male idiot of 41 paralyzed on one side both showed strabismus and sug gestion of microcephaly The next report was Fiocco (31) in 1913 he had observed this scalp disorder in a male cretin. Three years later Ganser (36) added two more cases, both microcephalic idiots one was epileptic and showed strabismus, and the other showed rotatory nystagmus. The next two cases were recorded by Galant (35) in 1918 one m 53-year-old male imbecile w th adiposogenital dystrophy and acromegaly and another m 65-year-old male idiot.

The first to report cutis verticis gyrata in mentally deficient woman was Anderson (6) in 1928. The woman was then 43 and no one knew when she first got the disorder Microscopic study only showed non-specific hypertrophy of the dermis. No other physical abnormality was present.

I 1929 Truffi (105) presented five more cases of cutis verticis gyrata in mentally deficient persons. One was severly retarded microcephalic girl of 11 who was unable to stand or walk. Three were severly retarded men 2 of them were epileptic and had slightly smaller heads than normal and the third was severely microcephalic. The fifth, slightly retarded man, was said to have an uneducable microcephalic brother who also had cutis erticis gyrata.

I 1933 Hellerstrom (47 described an interesting case of cutis erticis gyrata in 20-year-old male imbecile in which the scalp disorder disappeared after castration Besides the scalp disorder the patient had acanthosis nigricans and diabetes mellitus (Mescher 70 observed the same triad in man of normal intelligence

I 1938 Touraine and Gole (104 described two men with mild mental deficiency and th cut erticis gyrata apparently due to infection no neurologic disorders could be found in these men

I 1940 Radner 85 reported case of cutis erticis gyrata in 27-year-old male idiot with epilepsy and microcephal the man also had congenital nystagmus, strabismus and bilateral keratoconus.

In 1953 Polan and Butterworth (82) gave an exhaustive review of the literature on cutis verticis gyrata and also related six instances of the disorder in mentally deficient men. All six were idiots. Two had epilepsy One had spastic diplegia and congenital cataract. In another there was rotatory nystagmus, strabismus and unilateral cataract, and roentgenograms of the skull showed enlargement of the sella turcica. I three cases t was known when the scalp disorder was first seen in all three t was not until after puberty

In 1938 Kratter (55) recorded three cases of the scalp disorder in male microcephalic idiots tw of them had spastic diplegia. The following year Saetra (90) reported case of cutis verticis gyrata, endocrinopathy and mental deficiency in schizophrenic male this patient showed primary genital infantilism, w th only sparse hair on the pubis and armpits he got the scalp disorder after he was 20. Finally in 1962 Berg and Windrath-Scott (13) described male microcephalic idiot with cutis verticis gyrata and tetraplegia the scalp disorder was not noted until after the man had passed puberty

Material and methods

The Swedish series I analyzed consists of three groups of patients.

The largest group, 37 cases, consists of all the mentally deficient persons with cutis verticis gyrata registered at special schools and other Swedish institutions for the mentally deficient on Jan. 1 1962. They were collected by asking the physicians ttending these institutions to look for every case of the scalp disorder among the inmates and pupils they were told exactly what it was like and to palpate the scalp of every subject so as not to overlook any mild cases. After all the cases had been assembled, I mad physical examination of each subject whenever this was possible, and classified them according to the Vineland Social Ma urity Scale most of them being too retarded for an ordinary intelligence test.

The second group consists of 4 patients hom I examined in 1960 and 1961 who died before Jan 1 1962 and who are therefore not included in the census group.

The third group consists of 6 other patients who died before Jan. 1 1962 I got the data on

Table I Age distribution of 37 mentally deficient persons with cutis verticis gyrata on day of census (Jan. 1 1962)

Age (yrs)	No. of persons
20—25	2
25—30	4
30—35	8
35—40	7
40—45	6
45—50	6
50—	4

these cases from Prof. Hans Forssman of Uppsala.

During the years 1955 to 1959 Prof Hans Forssman made an inventory of all the patients and pupils enrolled in Swedish institutions for the mentally deficient on behalf of the Swedish Medical Board. During his examination of these altogether 12 903 persons, he found and examined 31 persons with cutis verticis gyrata. Thus I was able to check the observations I made in most of the first two groups of patients with what he had previously written about these patients. I am also indebted to his study for figures enabling me to calculate the incidence and mortality of this scalp disorder in Sweden

Whenever I examined a patient, I also read through all the medical records that could be obtained on his or her case. Most of the subjects had been institutionalized at an early age and I was able to follow the condition of several patients over the course of many years.

Epidemiologic observations

Prevalence and incidence

On Jan 1 1962 37 mentally deficient persons in Sweden had cutis verticis gyrata, 36 males and 1 female From this it may be estimated that the prevalence of a combination of mental deficiency and cutis verticis gyrata among Swedish males amounted to about 1×10^{-6} and among Swedish females to $2\ 6\times10^{-7}$

During the years 1955—1962, 8 new cases of cutis verticis gyrata developed among the mentally deficient population in Sweden. In addition to these 8, about which there was no doubt, there were 5 which might have started during these years Judging from this one or two new cases develop in this population every year It may be estimated therefore, that the incidence of a combination of cutis verticis gyrata and mental deficiency among Swedish males lies somewhere between $0\ 4\times10^{-6}$ and $0\ 6\times10^{-6}$

In a previous field study (4) I reckoned that severe mental deficiency with I Q s of 50 and under was present in about 0.5 per cent of each sex. At this rate, there would have been about 19 000 severely retarded men in Sweden on Jan. 1 1962 Assuming this, the prevalence of cutis verticis gyrata among severly mentally deficient men amounted to approximately 0.2 per cent. If it prevailed at the same rate among persons of normal intelligence, about 7,500 of them would have this scalp disorder So it is obviously much more common among the mentally deficient.

A combination of cutis verticis gyrata and mental deficiency probably occurs more frequently than appears from the foregoing figures for they are based only on institutionalized subjects However as persons of very low intelligence with severe physical disabilities are seldom tended at home in Sweden (4) it is not likely that the true figures are much higher

Geographical distribution

When 46 patients were grouped according to the county in which they were born it was shown that the birthplaces were spread fairly evenly all over Sweden (City of Stockholm 4 Stockholm 2 Upp-

sala 1 Östergötland 1 Jönköping 1 Kronoberg 1 Kalmar 2, Gotland 1 Kristianstad 1 Malmöhus 10, Halland 1 Älvsborg 3 Skaraborg 1 Värmland 2, Örebro 1 Västmanland 2, Kopparberg 3 Gävleborg 5 Västernorrland 2 Norrbotten 2) The 47th subject was born outside of Sweden.

Thirteen subjects, or 28 per cent, were born in towns and 10, or 21 per cent, were registered in towns when they were examined. This is a much lower proportion of town dwellers than in the general population. The reason is probably that there are far more severely retarded persons in the country than in the towns, due to a form of selective migration (6) This probably does not affect the observations in this study

Age of onset

Table I shows the ages of the 37 subjects in the census group they varied between 20 and 60, and the average was 38.3 years.

When a person with cutis verticis gyrata is mentally deficient it is nearly always impossible to find out when it began It always starts insidiously and these people are not as observant as others. Truffi (103) saw the disorder in a mentally deficient girl of 11 but most of the subjects reported have been between 20 and 60 years of age

It is known for certain that 8 of the patients in this series got the disorder between 1955 and 1962 when they were between 14 and 43 years of age. Thus the average age of onset in these 8 was approximately 31.5 years. Judging from the available information for the other cases, in none of them did the disorder develop before puberty The earliest observed was occurrence at the age of 17 in one patient.

Cutis verticis gyrata has been seen at birth, but then it is nearly always due to nevi (14, 41 50 53 73 81) and this secondary form is of no interest in the present connection.

Mortality

To get an idea of the prognosis of cutis verticis gyrata, I studied the 31 cases which Prof. Forssman had found and examined in his 1955—1959 survey

To begin with, I determined the mortality up to Jan. 1 1962. For this I used the method described by Larsson and Sjögren (58) which compares the mortality in the population under study with that in the corresponding normal population. Using this method, one divides the number of years the persons studied survived during the period of observation by the number of years an equal number of persons of exactly the same age could be expected to survive if the quotient amounts to 1 one assumes that the mortality in the two groups is the same if it amounts to 0.90, one assumes that the group under observation survive 90 per cent of the years survived by the normal population, and that the mortality among them is about 10 per cent higher than in the normal population.

The quotient for the present series amounted to 0.81 In other words, these 31 persons appeared to have an almost 20 per cent shorter life expectancy than the normal population during the same period

Large samples of the mentally deficient population in Sweden have shown a mortality about 30 per cent higher than in the normal population (5 58) It is hardly likely that mentally deficient persons with cutis verticis gyrata live longer than ones without. The reason for



Fig 1 Severe form of cutis verticis gyrata.

the difference is probably that the mortality for the cutis verticis gyrata series was calculated from a comparatively smaller series and over a comparatively shorter period of time.

As many as 11 of the 31 subjects died before the end of the period studied They all died of intercurrent infection of pneumonia and bronchopneumonia. They lived for 38 1 years, on the average

Judging from the present series, there fore, when cutis verticis gyrata develops in the mentally deficient, it does so late in life.

It would appear that every year in Sweden one or two mentally deficient persons with this disorder die and every year one or two more get the disorder In other words, the prevalence in Sweden of this combination seems to remain fairly constant.

Clinical observations

The folding of the scalp varied in appearance from case to case. As a rule, it was situated on the vertex, but it was not infrequently confined to one temporal region. Occasionally it was situated only on the frontal or occipital region. The folds either ran parallel to each other sometimes with and sometimes without anastomoses between them, or they radiated toward a common centre.

In severe cases, fifteen or more ridges could be counted and they were often 10 to 15 cm long and 0.5 to 1 cm wide. Though the hair nearly always hid some of the corrugation when the corrugation was severe the patients often looked ex tremely grotesque (fig 1)

At the other end of the scale were the mild cases in which one could barely see the folding and the ridges were few in number (fig 2)

Between these two extremes were cases of all degrees.

Mental abnormalities

As far as I could judge from testing and observation only 3 of the 47 patients had I Q s above 30 or 35

Twenty-six of the remaining 44 were quite shut off from other people they could not talk or eat or clothe them selves, they were incontinent of both urine and feces, and they could not be put to do anything purposeful The other 18 could communicate a little with the people around them they could obey simple commands and say simple words, they recognized some of their warders and they could be kept busy a little however they were not able to do even the simplest kind of work, they could not read or write as much as one letter of the alphabet and they all needed constant supervision All 44 were realized to be

tarded at an early age, and none had ver gone to school. They seemed to iffer from a form of stationary idiocy one showed any signs of deterioration.

The remaining 3 subjects were only lightly retarded, having I Q s between about 60 and 70 Two of the 3 probably lso had catatonic schizophrenia and the hird showed adiposogenital dystrophy with an undescended testis.

Other physical abnormalities

The patients were not so alike in the nature of other physical abnormalities accompanying the scalp disorder as they were in the type of mental retardation. Most of their other physical abnormalities however fell into three groups epilepsy cerebral palsy and eye defects. Nine, or 19 per cent, suffered from all three, 13 or 28 per cent, from two and 16, or 34 per cent from one of these types of disorder Only 9 had none of these three forms of disorder

Epilepsy No less than 26, or 55 per cent of the patients, had or had had seizures of the grand mal variety the corresponding frequency for all persons with severe undifferentiated mental deficiency has been estimated in field studies to be between 5 and 10 per cent (4 43) Twenty-one were still having seizures when they were examined. Two had not had any since they were 2.

In 22 cases t was known when the epilepsy began In 8 of these t was before the age of 2, in 9 between the ages of 2 and 5 and in 5 after the age of 5. This is in keeping with the age distributions found in large series of severely retarded persons with epilepsy 51)

Thus epilepsy is much more common among mentally deficient persons with cutis erticis gyrata than among all persons with undifferentiated mental



Fig. 2. Mild form of cutis verticis gyrata.

deficiency On the other hand, epilepsy seems to begin t the same ages in the cases of cutis verticis gyrata as it does in the larger group.

Cerebral palsy Twenty-five, or 53 per cent, of the patients in the present series had cerebral palsy all 25 suffered from severe spastic paralysis, and 11 were bedridden with secondary contractures in their legs. In comparison it is calculated that only 2 or 3 per cent of all mentally deficient persons suffer from spastic paralysis (4 43)

Eleven patients had paraplegia, 9 tri- or tetraplegia, 4 hemiplegia and 1 diplegia. Four of the 25 also showed thetosis and 2 taxia. Non of them were born prematurely

Eye defects Si patients were totally blind 11 showed strabismus, 7 cataract, 4 nystagmus and 2 keratoconus.

Altogether 21 patients had one or more eye defects.

Other abnormalities As seen from the survey of the literature, the patient was said to be microcephalic in most of the reported cases of cutis verticis gyrata in combination with mental deficiency As has been pointed out (17 24) however some authors use the word microcephalic far too loosely According to the manual on terminology and classification published by the American Association on Mental Deficiency in 1959 microcephaly is considered to be present when the circumference of the head does not exceed 42 cm at adult age If all the authors describing patients with cutis verticis gyrata and mental deficiency had kept to this definition they would not have called so many of them microcephalic. Persons with a combination of cutis verticis gyrata and mental deficiency probably fall into two groups a large number with slightly smaller heads than normal and a small number who are truly microcephalic.

In 12 of the present cases, the head was 52 cm or less in circumference in 8 between 50 and 52 cm in 2 between 47 and 50 cm and in 2 it was 47 cm or less

Thus severe microcephaly cannot be a common concomitant of cutis verticis gyrata.

Persons with severe mental deficiency of unknown origin are often underdeveloped on the whole Davies and Kirman (26) for example, noted that 25 per cent of persons with severe mental deficiency of unknown origin had a skull circumference below three times the standard deviation for the normal population Thus a small head need not be a particular characteristic of mentally deficient persons with cutis verticis gyrata

The skull was roentgenographed in 11 of the present cases and the hands and feet in 6 *Only 1 patient showed a* roentgenographic feature of note a greatly enlarged sella turcica this patient also showed clinical signs of acromegaly Three other patients showed acromegaly like features. One of these patients probably also had catatonic schizophrenia (Finkelstein (30) described a case of catatonic schizophrenia acromegaly and cutis verticis gyrata) This patient s head was 60 cm in circumference and both his face and limbs were enlarged his feet were so large that he had to have his shoes made to order His sella turcica was of normal shape (as it also was in Finkelstein s case)

Four patients were abnormally short they were only 158 154 150 and 142 cm tall.

Six patients showed kyphosis, sometimes combined with scoliosis.

Talipes equinovarus was noted in 6 patients flatfoot in 6 and funnel chest in 4 Phenylketonuria was noted in 1 patient, where it was probably only coincidental.

Adiposogenital dystrophy and undescended testis was observed in 1 case this is interesting in view of Galant s (35) case of adiposogenital dystrophy mental deficiency and cutis gyrata All the rest of the men showed normal primary and secondary sex characteristics. Several of the men had also been observed to have ejaculations.

Consanguinity

None of the patients parents were more closely related than first cousins. According to the genealogic data in the parish records 2 patients were the children of first cousins 21 were definitely not and 1 was probably not so In 23 cases not enough was known about the parents to be able to tell whether they were related or not. I estimated therefore that about 8 per cent of the patients were

the offspring of first cousins, as opposed to 1—2 per cent of the normal population in Sweden (4).

Laboratory data

Sex chromatin and chromosomes

Because of the abnormal preponderance of males in the series, the sex chromatin of 14 men was studied with the buccal smear method of Klinger and Ludwig (54). None of the 14 showed sex chromatin bodies.

Analysis of the chromosomes in the peripheral leukocytes of 1 patient showed a normal male karyotype and no abnormality in the chromosomes.

Chromatographic urinanalysis

The urine of 12 patients was analyzed by ascending two-dimensional paper chromatography using no. 1 Whatman filter paper. N-butanol-acetic acid-water (4+1+2) was used for the solvent in the first direction and n-butanol methyl-ethylketone-water (2+2+1) with an atmosphere saturated with cyclohexylamine in the second. The papers were dried at 80 C then dipped in a solution of 0.2 % ninhydrin in acetone containing 7 % glacial acetic acid, and heated for 15 min. at 55 C.

No qualitative abnormalities were seen in these samples. Nor did quantitative paper electrophoresis at a pH of 3.6 reveal anything abnormal in the quantity of beta-amino-isobutyric acid excreted.

Hormone analysis

The urine of 8 patients was examined for hormones (table II).

As will be seen, most of the patients excreted only small amounts of total gonadotropins, 17-ketosteroids and 17-hydroxycorticosteroids. One patient did not even excrete a measurable amount of luteinizing hormone (LH).

Table II. Hormone studies

Age (yrs)	Total gonadotropins (Mouse units/24 hrs)	17 hydroxy-corticosteroids (mg/24 hrs)	17-ketosteroids (mg/24 hrs)
49	~ 6	1.4	1.3
43	> 6<12	6.3	8.1
33	~12	8.0	2.4
31	< 6	—	—
44	< 6	16.0	9.2
23	>12<60	7.5	4.8
26	< 6	3.7	1.5
51	<12	4.0	1.8

While no definite conclusions can be drawn from this small sample, the results point to dysfunction of the pituitary gland.

Discussion

Several different origins have been suggested for primary cutis verticis gyrata. There has been a great amount of speculation and I shall not give any account of it here as many others have already done so (32, 82, 94, 97, 98, 105, 115). It is very likely that cutis verticis gyrata can be brought about by several different mechanisms. To my mind, a plausible explanation for many of the primary cases is a disorder in endocrine function. This explanation is borne out by five circumstances: the sex distribution, the age of onset, the occurrence together with acromegaly, acromegaly-like conditions and primary sex infantilism, the effect of castration and hypophysectomy, the preliminary results of hormone analysis.

Other abnormalities As seen from the survey of the literature, the patient was said to be microcephalic in most of the reported cases of cutis verticis gyrata in combination with mental deficiency As has been pointed out (17 24) however some authors use the word microcephalic far too loosely According to the manual on terminology and classification published by the American Association on Mental Deficiency in 1959 microcephaly is considered to be present when the circumference of the head does not exceed 42 cm at adult age. If all the authors describing patients with cutis verticis gyrata and mental deficiency had kept to this definition they would not have called so many of them microcephalic. Persons with a combination of cutis verticis gyrata and mental deficiency probably fall into two groups a large number with slightly smaller heads than normal and a small number who are truly microcephalic

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tary gland was removed. Irradiation of the pituitary gland had no effect on the scalp in Foerster's and Wider's case (33) of the same combination, however. Nor did thyroid compounds have any effect in Grönberg's case (42).

Results of hormone analysis. The results of the pilot study of the hormones in the urine of 8 of the present subjects pointed to an endocrine background for their scalp disorder.

Judging from the present cases, mentally deficient patients with cutis verticis gyrata often suffer from epilepsy, cerebral palsy and eye defects. 38 of the 47 patients had one or more of these disorders. It is impossible as yet, however, to classify the different clinical groups into different etiologic entities.

It would appear that a combination of cutis verticis gyrata and mental deficiency may occur in syndromes of several different origins. Most commonly, however, the combination seems to be part of a syndrome which in complete form is also characterized by epilepsy, cerebral palsy and eye defects.

Summary

Cutis verticis gyrata and mental deficiency is a rare combination, and almost nothing has been written about its epidemiologic characteristics or learnt about its origin. Because of this, I analyzed 47 Swedish cases of the combination, 46 in males and 1 in a woman. I made the following observations.

The prevalence of the combination among males in Sweden amounts to approximately 1×10^{-4} and the incidence to somewhere between 0.4×10^{-4} and 0.6×10^{-4}. The birthplaces of the patients were distributed fairly evenly all over the country. In none of the patients did the scalp disorder develop before puberty. The patients who died during the period studied had lived for 38.1 years on the average. The number of new cases per year in Sweden (1—2) roughly corresponded to the number of patients who died.

Forty-four of the 47 patients were severely retarded. Fifty-five per cent had epilepsy, 53 per cent cerebral palsy and 45 per cent eye defects.

About 8 per cent were the children of first cousins.

Studies of the sex chromatin and chromosomes, and chromatographic analysis of the urine gave normal results. A pilot study of the hormones in the urine pointed to endocrine disorder.

Four other circumstances indicate that cutis verticis gyrata is caused by an endocrine disorder: the disproportion between the sexes, rare onset before puberty, frequent combination with acromegaly, acromegaly-like conditions and primary sex infantilism, beneficial effects of castration and hypophysectomy.

Acknowledgements

The author is much indebted to Prof. H. Forssman at the Psychiatric Research Centre, Ulleråker, Uppsala, who generously let me use his own material on this subject.

The hormone analyses were done at the Hormone Laboratory at the Academic Hospital in Uppsala under the direction of Dr. B. H. Persson, and for this I am very grateful.

My thanks are also due to Drs. K. H. Gustavson and L. G. Lundin at the Institute for Medical Genetics, University of Uppsala, who did the chromosome study and the chromatographic urine analysis.

The study was supported by a grant from the Nilsson-Ehle Fund.

Sex distribution Cutis verticis gyrata seldom occurs in women It used to be thought that it was often overlooked in women because of the way they do their hair but the present study has shown that it really is rare among women

It is interesting that the only woman in the present series was abnormally hairy This subject aged 35 was normally delivered after an uneventful pregnancy It was obvious when she was only an infant that she was retarded and she was sent to an institution for the mentally deficient early in life. At the time of the present study she could not talk could not be made to do anything meaningful could not dress herself did not recognize her relatives and needed constant watching Her I Q was probably no more than 20 (Her social age on the Vineland Social Maturity Scale amounted to 2 4 years.) Physical examination showed extreme hirsutism, spastic paraplegia, bilateral keratoconus and mild strabismus. She had never had any epileptic attacks. Her external genitals and breasts were normally developed and she menstruated normally The cutis verticis gyrata did not begin until she was over 27

Age of onset No author has ever reported cutis verticis gyrata before puberty in a mentally deficient person and there was no case in the present series. On going through the 185 cases of normal intelligence in the literature to which I had access I found only 22 instances in which the scalp disorder began before puberty and in 18 of these 22 the scalp disorder was of secondary nature it was due to a nevus in 15 and to infection in 3 This almost invariable onset after puberty points to endocrine origin

Frequent occurrence together with acromegaly acromegaly-like conditions and primary sex infantilism The literature contains several reports of cutis verticis gyrata combined with acromegaly and acromegaly-like conditions Altogether 9 per cent of the present patients had acromegaly-like features. It may be that pachydermoperiostosis is also caused by hormonal dysfunction however it is still not known how this familial disorder is caused. Cutis verticis gyrata has also been found in combination with primary sex infantilism, another manifestation of endocrine disorder (77 90 92) In Galant s case it was combined with adiposogenital dystrophy and the same was true in one of the present cases.

Effect of castration and hypophysectomy Hellerström (47) described a case of cutis verticis gyrata, acanthosis nigricans and diabetes mellitus, in which the scalp disorder disappeared after castration. The same happened in Yamagata s case (110) this was a man of 47 of normal intelligence who had had cutis verticis gyrata since he was 20 a week after castration the corrugation on his scalp showed signs of receding and six months after the operation it had vanished completely

Because of these observations on the effect of castration, I tried the effect of large amounts of oestrogenic compounds by mouth on two patients. Using a dosage of 1 mg of ethinyl estradiol (Linoral manufactured by the Pharmacia Drug Co) per day I treated one patient for two weeks and after an interval of a month for four and a half months again, and another patient for two weeks and after an interval of a month for another five weeks. In neither case did the treatment lead to any definite improvement.

Serfling (93) recently described a case of acromegaly and cutis verticis gyrata in which the scalp disorder vanished when an eosinophilic tumor in the pitui

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Vom 8 bis 11 April 1964 findet in Bad Nauheim, Köln, der Kongreß der *Deutschen Gesellschaft für Bluttransfusion* statt

Hauptthemen

1) Serologische Vorbereitung der Bluttransfusion (Podiumsdiskussion)
2) Technische Probleme der Blutkonservierung (Podiumsdiskussion)
3) Der hämolytische Transfusionszwischenfall mit Berücksichtigung der extrakorporalen Dialyse.
4) Die Indikation der gezielten Bluttransfusion.
   a) Die Indikation der Bluttransfusion
   b) Die Blutkomponenten Therapie
5) Serologie und Praxis der Austauschtransfusion.

Interessenten erhalten auf Anfrage genauere Informationen durch Dr med Orth, 63 Gießen 2 Postfach 2629 Vortragsanmeldungen werden nur durch Professor Dr Dahr 5 Köln Höhenberg Postfach 48 entgegengenommen

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The Fifth Congress of the International Diabetes Federation will be held in Toronto, Canada, during July 20—24 1964 The American Diabetes Association is taking part in the Fifth Congress in place of its regular postgraduate program and annual meeting in 1964 The Congress is being held under the joint sponsorship of the Canadian Diabetic Association and the University of Toronto

The entire program is so arranged that all concerned with diabetes should find an area of particular interest

Information concerning the congress including registration and abstract forms, is enclosed in a brochure which will be sent upon request. Such requests should be mailed to The Executive Secretary International Diabetes Federation Fifth Congress 477 Mount Pleasant Road Toronto 7 Ontario Canada

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8th International Congress of Internal Medicine November 23rd—28th 1964 Buenos Aires (Argentina)

President of the Congress Prof Mariano R. Castex, Zabala 2126 Buenos Aires.

Principal subjects of the congress

1 Chromosomes and pathology
2 The internist. Importance Scientific moral and intellectual conditions.
3 Iatrogenic diseases.
4 American geographic pathology (Chagas mycosis Junin s disease)
5 Paraneoplasic syndromes (including quantitative and qualitative disturbances of the plasmaproteins in neoplasies)

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the National Organisation for Health Research, Toegepast Natuurwetenschappelijk Onderzoek, Applied Scientific Research (President J W Tesch, M. D)
The Hague, the Netherlands

A Poliomyelitis Epidemic in Statu Nascendi

By

J J van Loghem

In non-tropical countries in the Northern and Southern hemispheres, the incidence of poliomyelitis usually reaches its lowest point in early spring this is followed by the pre-epidemic stage of a new epidemic.

This parasynchronism has suggested the presence of an exogenous, climatic factor governing the seasonal variation of the commensal symbiosis between man and poliomyelitis virus (1)

I now wish to draw special attention to the behaviour of poliomyelitis virus during the period when, as appears from the pre-epidemic increase in the number of cases, the exogenous factor arouses the virus from its latency

The question may be approached by analysing the statistical data on the numbers of cases notified and the way in which the epidemic dies away and later redevelops in the sub-regions of the regio epidemica.

For this purpose I studied the post-epidemic, inter-epidemic and pre-epidemic figures for the first 6 months of 1959 in the large territory of the German Federal Republic. They were derived from the "Schnellmeldungen" and Nachmeldungen" (notifications) of the Bundesgesundheitsamt (Federal Health Department) in Berlin. In these weekly reports the cases are noted according to the "Länder" "Regierungsbezirke" and Kreise in which they have occurred.

Table I surveys the cases notified in the seven German "Länder" — North Rhine-Westphalia, Bavaria, Lower Saxony Baden-Württenberg, Hessen, Rhineland-Palatinate and Schleswig Holstein — during the first seven 4-weekly periods of 1959 In this period the 1958 epidemic ended and the 1959 epidemic began to develop.

It will be observed that the cases diagnosed in the 4-weekly periods — 30 22 12, 18, 18, 50 137 — cannot have been due to the existence somewhere in Germany of a "focus" of infection which might have been nearly extinguished and then flared up again. In spite of their small number the cases were spread quite evenly among a population of over 49 000,000.

The following particulars have been arranged according t the seven 4-weekly periods (I—VII)

Submitted for publication June 26, 1963.

Table I Absolute numbers of poliomyelitis cases in the first seven 4-weekly periods of 1959 in seven "Länder" of the German Federal Republic

"Länder" (population in mill.)		I	II	III	IV	V	VI	VII
		4/1—31/1	1/2—28/2	1/3—28/3	29/3—25/4	26/4—23/5	24/5—20/6	21/6—18/7
North Rhine-Westphalia	(15.6)	7	6	2	2	7	15	26
Bavaria	(9.3)	8	8	5	5	8	24	50
Lower Saxony	(6.5)	5	3	2	3	1	2	15
Baden-Württemberg	(7.5)	4	1	2	3	1	3	31
Hessen	(4.7)	2	0	0	1	0	1	2
Rhineland Palatinate	(3.4)	2	2	0	3	1	4	9
Schleswig Holstein	(2.3)	2	2	1	1	0	1	4
	Total	30	22	12	18	18	50	137

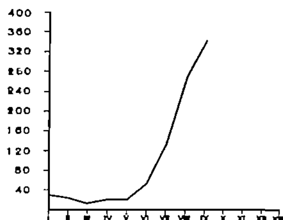


Fig 1 Poliomyelitis number of cases in the German Federal Republic during the first 6 months of 1959

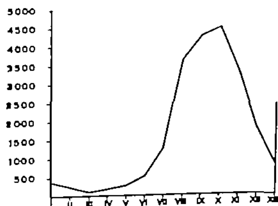


Fig 2 Poliomyelitis number of cases in the German Federal Republic during the period 1952—1956.

4-weekly periods I and II (post-epidemic stage)

The figures for the periods I (January 4—31) and II (February 1—28) refer to 30 and 22 cases respectively post-epidemic remnants from 1958. These cases were spread over all seven of the "Länder" and had been reported from 44 Kreise" of 23 "Regierungsbezirke As the seven Länder" have altogether 33 Regierungsbezirke (there are no Regierungsbezirke" in Schleswig-Holstein) it means that a very great part of the Federal Republic was involved in the 52 cases. Their even distribution is also apparent from the number of inhabitants and the distribution over the various Kreise Of the 44 Kreise 12 were in North Rhine-Westphalia and 12 in Bavaria, two Länder" with a total population of 24.9 million. In the five Länder" with a total population of 24 4 million, cases were notified in 20 Kreise

It may be concluded, therefore, that during the first two months of 1959 shortly before the inter-epidemic lowest point, enough virus was circulating in two-thirds of the territorial subdivisions of the Federal Republic to give rise to sporadic cases of poliomyelitis. *No* direct connections between these cases could be established

4-weekly period III (inter-epidemic stage)

Table I and fig 1 show that the number of cases was at its lowest in period III (March 1—28 1959) when 12 cases were notified. Figs. 1 and 2 demonstrate that the 1959 epidemic

Table II Number of "Kreise" by which the epidemic region of the German Federal Republic was increased (from 10 to 123) during the five 4-weekly periods III—VII of 1959 For absolute numbers of cases see table I

"Länder" and "Regierungsbezirke" in which cases were reported (total no. in parenthesis)	III	IV	V	VI	VII
	1/3—28/3	29/3—25/4	26/4—23/5	24/5—20/6	21/6—18/7
	No. of "Kreise" with cases	No. of "Kreise" where cases were notified for the first time in the 4 pre-epidemic 4-weekly periods			
North Rhine Westphalia (6)					
Münster Arnsberg, Cologne, Düsseldorf, Detmond	2	2	6	5	8
Bavaria (7)					
Upper Bavaria, Lower Bavaria, Upper Palatinate Upper Franconia, Central Franconia, Lower Franconia, Swabia	3	4	6	12	13
Lower Saxony (8)					
Hannover Lüneburg Stade, Osnabrück, Aurich, Oldenburg, Hildesheim	2	2	1	1	15
Baden-Württemberg (4)					
North Württemberg, North Baden, South Baden, South West Hohenzollern	2	2	1	3	14
Hessen (3)					
Darmstadt, Wiesbaden	0	1	0	1	2
Rhineland-Palatinate (3)					
Treves, Coblenz, Palatinate, Montabaur	0	3	1	2	3
Schleswig-Holstein (0)	1	1	0	1	0
No. of "Kreise" in periods III—VII	10	25	40	65	123

course of poliomyelitis corresponds to the typical seasonal variation observed in the German Federal Republic in the preceding years. The 1952—58 statistics were derived from the Epidemiological and Vital Statistics Reports of the World Health Organisation, Geneva.

The distribution of the 12 cases over 5 of the 7 "Länder" was as follows North Rhine Westphalia 2, Bavaria 5, Lower Saxony 2, Baden-Württemberg 2, Schleswig-Holstein 1 The cases occurred in 9 "Regierungsbezirke" and 10 "Kreise" Thus the even distribution remained even in this last post-epidemic remnant. Of the 10 "Kreise" involved in this period, 5 had not been mentioned in periods I and II. This indicates that in the post-epidemic stage, virus circulation prevailed over a wider area than the actual cases.

4-weekly periods IV and V (pre-epidemic stage)

The 12 cases of period III were followed by 18 cases in period IV (March 29—April 25)

Table I Absolute numbers of poliomyelitis cases in the first seven 4-weekly periods of 1959 in seven "Länder" of the German Federal Republic

"Länder" (population in mill.)		I	II	III	IV	V	VI	VII
		4/1—31/1	1/2—28/2	1/3—28/3	29/3—25/4	26/4—23/5	24/5—20/6	21/6—18/7
North Rhine Westphalia	(15.6)	7	6	2	2	7	15	26
Bavaria	(9.3)	8	8	5	5	8	24	50
Lower Saxony	(6.5)	5	3	2	3	1	2	15
Baden Württemberg	(7.5)	4	1	2	3	1	3	31
Hessen	(4 7)	2	0	0	1	0	1	2
Rhineland Palatinate	(3.4)	2	2	0	3	1	4	9
Schleswig Holstein	(2.3)	2	2	1	1	0	1	4
	Total	30	22	12	18	18	50	137

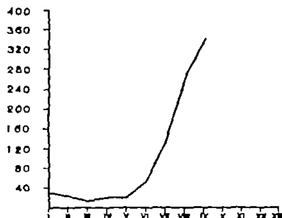


Fig 1 Poliomyelitis number of cases in the German Federal Republic during the first 6 months of 1959

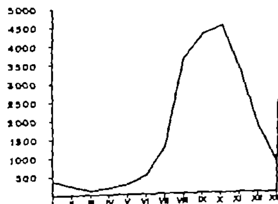


Fig 2 Poliomyelitis number of cases in the German Federal Republic during the period 1952—1956.

4-weekly periods I and II (post-epidemic stage)

The figures for the periods I (January 4—31) and II (February 1—28) refer to 30 and 22 cases respectively post-epidemic remnants from 1958. These cases were spread over all seven of the "Länder" and had been reported from 44 "Kreise" of 23 "Regierungsbezirke". As the seven "Länder" have altogether 33 "Regierungsbezirke" (there are no "Regierungsbezirke" in Schleswig Holstein) it means that a very great part of the Federal Republic was involved in the 52 cases. Their even distribution is also apparent from the number of inhabitants and the distribution over the various "Kreise". Of the 44 "Kreise" 12 were in North Rhine-Westphalia and 12 in Bavaria, two "Länder" with a total population of 24 9 million In the five "Länder" with a total population of 24 4 million, cases were notified in 20 "Kreise"

It may be concluded, therefore, that during the first two months of 1959 shortly before the inter-epidemic lowest point, enough virus was circulating in two-thirds of the territorial subdivisions of the Federal Republic to give rise to sporadic cases of poliomyelitis. No direct connections between these cases could be established

4-weekly period III (inter-epidemic stage)

Table I and fig 1 show that the number of cases was at its lowest in period III (March 1—28, 1959) when 12 cases were notified. Figs. 1 and 2 demonstrate that the 1959 epidemic

with the seasons. This results in variation of the virus circulation rate and the incidence of infection.

Nor do we know anything about the nature of the exogenous factor. We can only conclude from the alternating synchronism of poliomyelitis in the two hemispheres that this exogenous factor must be climatic.

It is also significant that the pre-epidemic onset of poliomyelitis in non-tropical countries of both hemispheres coincides approximately with the vernal equinox, i. e. the time when the days begin to get longer than the nights. This is consistent with the fact that near the equator where day and night are of approximately equal length, there is no seasonal variation in the incidence of poliomyelitis (2)

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which were spread over all 7 'Länder', and again by 18 in period V This would seem to indicate that the pre-epidemic stage had set in.

The first onset of the seasonal increase is more apparent from the higher number of Kreise involved than from the number of cases, as will be seen from table II For instance to the two Kreise in North Rhine Westphalia mentioned in period III 8 (2 and 6) new Kreise had been added in periods IV and V Similar increases during periods IV and V may be found for Bavaria (10) Lower Saxony (3) Baden-Württemberg (3) Hessen (1) Rhineland Palatinate (4) and Schleswig Holstein (1) Thus as many as 30 Kreise" were added in the early pre-epidemic stage to the 10 at the lowest point (period III)

The pre-epidemic increase may then not be obvious from the small numbers of cases, but it will become apparent from a study of the number of Kreise in which they occurred. I shall revert to this subject in the discussion of this report

4-weekly periods VI and VII

In most countries in the Northern hemisphere, the pre-epidemic stage ends during the periods VI and VII (May 24—July 18) Table I shows that in 5 of the 7 Länder' a fairly strong epidemic development had set in by period VII I refer again to table II, which shows the increase in Kreise" from which the first cases were reported in the preepidemic stage of 1959

In the periods III—VII altogether 235 cases were reported in 123 Kreise Obviously the sporadicity of the cases was maintained when the number of Kreise" increased

Discussion and summary

In the preceding pages we have drawn attention to a seasonal phenomenon the genesis of the poliomyelitis epidemic in West Germany in the first six months of 1959 During this period we have distinguished a final post-epidemic phase of the 1958 epidemic in January and February an inter-epidemic phase represented by the lowest point of the incidence curve in March and a pre-epidemic phase from April to July which preceded the epidemic appearance of the disease.

The extensive area in which the remnants of the dwindling 1958 epidemic manifested themselves was highly significant. The 52 sporadic cases diagnosed in January and February were scattered over 7 "Länder' in 44 "Kreise" of 25 "Regierungsbezirke" Such even distribution also characterized the 12 sporadic cases which marked the lowest point of the curve in March. They were observed in 10 "Kreise of 9 "Regierungsbezirke in 5 "Länder

It must be appreciated that neither the 52 in January-February group nor the 12 in March group — a total of 64 cases in a population of over 49 million — can be attributed to case-to-case transmission. Such a theory would not agree with the even distribution throughout the 7 "Länder" which persisted even when the number of cases declined everywhere towards the inter-epidemic low point.

The sporadic cases in the post-epidemic and inter-epidemic periods are better interpreted as indicators of the ubiquitous circulation of poliomyelitis virus, the excreted product of a symbiosis which is subject to the influence of an exogenous factor

It will also be observed that the even distribution of post-epidemic and inter epidemic cases is likewise seen in the pre epidemic phase. The 18 new cases of period IV and the 18 of period V were scattered over a rapidly increasing number of "Kreise

We have no conception of the way in which the exogenous factor could influence the intracellular existence of poliomyelitis virus in the human intestinal tract. We can only infer from the available data that the amount of symbiosis between man and virus fluctuates in close association

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From Nörre Hospital (Kobenhavns Plejehjem) (Head Knud Raby M. D.)
Copenhagen, Denmark

Cyclopenthiazide with and without Supplementary Potassium

Effects in Long-term Treatment of Patients with Negligible Excretory Elimination of Oedema

By

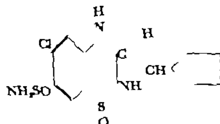
René Dybkær and Allan Frantzen

During the last few years the benzothiadiazines have proved their value as orally active diuretics of the saluretic type The main effect is probably a depression in the proximal segment of the renal tubule, of the reabsorption of sodium ions and consequently of chloride ions, and water The resulting increased flow through the distal segment facilitates the normal exchange of potassium ions and hydrogen ions, which pass from the blood to the urine, with sodium ions which pass from the urine to the blood this process is intensified by aldosterone. In consequence of the increased ion exchange the increased output of water is "complicated" by an increase of the potassium excretion often resulting in hypokalaemia. As the sodium/chloride ratio in the additionally excreted urine is 1 00 whereas the ratio in the extracellular fluid is about 1.2 a fall in serum chlorid ensues. Finally rise in serum standard bicarbonate reflects metabolic basosis, probably caused by an increased excretion of hydrogen ions (10)

Of these undesirable side effects the most serious one seems to be the alterations in potassium metabolism, especially since the intracellular potassium loss may be considerable despite a modest decrease in the serum potassium concentration (6 10) In cases with excretory elimination of severe oedema the disturbance of potassium balance is most marked.

In addition to the saluretic effect, the benzothiadiazines may also exert a moderate hypotensive action which may be of some value in the treatment of hypertensive patients, chiefly when used in combination with other antihypertensive drugs (2, 12)

One of the latest benzothiadiazines is cyclopenthiazide



(cyclopenthiazide (Navidrex®)

(6-chloro-3-cyclopentyl-methyl-3,4-dihydro 7-sulphamyl 1,2,4-benzothiadiazine 1 1-dioxide) which possesses a full diuretic effect in doses of one milligram and thus

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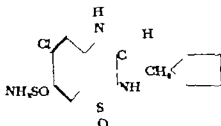
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may claim the highest water excretion effect/dose ratio yet encountered in a diuretic. In therapeutic doses the carbonic anhydrase inhibitor effect is negligible in human subjects (3)

Earlier clinical investigations on the effect of this potent drug have been concerned mostly with the diuretic effect in short term experiments on normals (3 13) or oedematous patients (4 10 13) or in long term experiments on cirrhotics or oedematous patients (10 13)

The effect of the long term treatment of non-oedematous non-cirrhotic patients (with or without hypertension) has been reported (12) — but without studies on the urinary excretion of electrolytes.

Apart from a passing remark (13) the literature does not seem to have brought any reports on long term cyclopenthiazide medication especially in subjects without hepatic cirrhosis or elimination of oedema It was decided therefore, to investigate this matter in order to obtain information on possible side effects during treatment with this drug in the aforementioned types of patients Furthermore a study of the corrective action — if any — of simultaneous orally administered potassium chloride might be of practical interest.

Material and methods

Five female, chronic patients in a steady state were selected, because their clinical condition appeared to permit a nearly quantitative collection of the 24-hour urine.

Briefly the clinical pictures were as follows

Case 1 71 years. Sequelae of stroke arteriosclerotic heart disease cardiac hypertrophy ECG normal arterial hypertension (B.P about 175/90 mm Hg) renal function normal the patient is chair bound. No oedema during the trial.

Case 2. 75 years. Sequelae of stroke X-rays of chest normal ECG shows left axis deviation arterial hypertension (B.P about 200/100 mm Hg) intermittent proteinuria, serum creatinine 97 μmol/l ($\sim$ 1 1 mg/100 ml). Rather immobile. Moderate ankle oedema without significant change during the trial.

Case 3. 80 years. Diabetes arteriosclerotic heart disease (cardiac hypertrophy no pulmonary congestion, ECG flattened T_1 wave) arterial hypertension (B.P about 175/75 mm Hg) no proteinuria, serum creatinine about 124 μmol/l ($\sim$ 1 4 mg/100 ml) The patient is immobile. Considerable crural oedema which did not change during the trial.

Case 4 75 years. Sequelae of stroke arteriosclerotic heart disease (angina pectoris, normal X rays of the chest, ECG normal) renal function normal B.P 140/75 mm Hg. The patient does not leave her chair Slight ankle oedema which did not change during the trial.

Case 5. 86 years. Arteriosclerotic heart disease (X-rays of the chest showed an enlarged aorta, a normal heart, and no pulmonary congestion ECG low T_1 wave) B.P about 120/75 mm Hg kidney function subnormal with serum creatinine 124 μmol/l ($\sim$ 1 4 mg/100 ml) but no proteinuria. Can walk a little with support. The moderately pronounced crural oedema showed little change during the trial, at least in relation to the medication.

Laboratory examinations

Every morning the 24-hour urine was collected and pooled. The following determinations were made Volume and specific gravity chloride concentration titrimetric by the method of Brun (1) sodium and potassium concentrations two or three different dilutions were measured on a Baird Atomic, Inc. direct reading flame photometer maintenance of level was ensured by re-determination of a specimen from the day before and repeated determinations on a deep-frozen urine specimen as well as on independently made aqueous standards.

Twice weekly Concentrations in serum of chloride potassium, sodium (determined on the same instrument as for the urine) and

total carbon dioxide (van Slyke apparatus)
Blood pressure.

Once week: Excretion of uric acid in 24-hour urine. Concentrations in serum of uric acid, creatinine, and total protein; in blood of urea, haemoglobin, and leucocytes in plasma of platelets.

Every two weeks (mostly): Body weight.

Before and after the experiment: ECGs.

Dosage of diuretic: Four patients received 1.00 mg cyclopenthiazide, divided into two equal doses, morning and noon, and later 1 00 mg cyclopenthiazide with 2,400 mg potassium chloride (equalling 32.2 mmol) similarly divided. One patient, having body weight of only 49 kg, received three quarters of these doses (equal doses morning, noon and after noon)

Plan. The experiment on each patient was planned for fourteen weeks, but unavoidable circumstances involved minor variations, for which appropriate corrections are made in the calculations. Only two of the courses were run nearly simultaneously (cases 1 and 5). The 14 "weeks" were divided as follows

C1 — preliminary control period of two weeks,

Cp — treatment with cyclopenthiazide for four weeks,

C2 — two-week interval without treatment,

CpK — four-week treatment with cyclopenthiazide and potassium chloride and, finally

C3 — period of two weeks without treatment.

For most laboratory data the weekly 24-hour means were calculated and, thus, each type of examination is represented by 2 + 4 + 2 4 + 2 mean values

Statistical calculation

Standard methods were used throughout, especially analysis of variance

Results and comments

Body weight

The weighings were regrettably not mad under strictly comparabl conditions, but no major variations were found the maximum loss was 1 9 kg, the maximum gain 1 4 kg, and a minor increase in body weight during treatment was found in several cases.

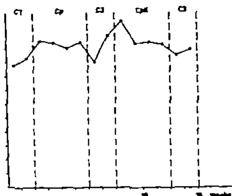


Fig 1 Volume of 24-hour urine. Weekly means for 5 patients. C1 preliminary control period, Cp treatment with cyclopenthiazide, C2 period of restitution, CpK treatment with cyclopenthiazide and potassium chloride, C3 final control period.

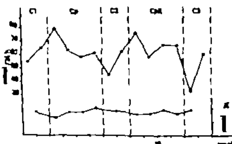


Fig 2 Urinary sodium and uric acid, 24-hour excretion. Upper curve weekly means of sodium output (5 patients) Lower curve weekly values of uric acid (two-week means are given for the 1st + 2nd and 7th + 8th week the values for the 14th week are missing)

Haemoglobin blood urea, and serum protein concentrations

The concentrations varied slightly during the treatment and not in a way suggesting haemoconcentration.

From these results and the periodic clinical examinations we conclude that no major excretory elimination of oedema occurred.

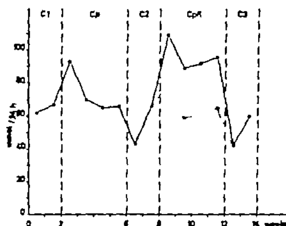


Fig 3 Urinary chloride 24-hour excretion. Weekly means for five patients. Squares represent values corrected for chloride added in tablets.



Fig 4 Urinary potassium, 24-hour excretion. Weekly means for five patients. Squares represent values corrected for potassium added in tablets.

Urine

The excretions of urine, sodium chloride, and potassium as well as the excretions of chloride and potassium corrected for the amount added in the tablets during the CpK period were calculated for each 24-hour period Comparisons were made for different lengths of time between the values before and after each shift of regimen e.g the means of chloride excretion for the last 24 48, and 168 hours of the Cp period were compared with the means of the corresponding first 24 48 and 168 hours of the C2 period Hereafter these periods of time will be designated 1 d 2 d, and 1 wk respectively Furthermore, the average 24-hour excretion in each of the 14 weeks was calculated.

Volume The 1 d and 2 d comparisons for the values at the transition from period C1 ⟶ Cp and C2 ⟶ CpK showed only moderate variations, but mostly a rise the 1 wk comparisons always showed an increase (fig 1) The changes at Cp ⟶ C2 and CpK ⟶ C3 were always a moderate decrease (except in one patient where a slight rise in the 2 d comparison was recorded) The weekly averages in the last three-quarters of the Cp period for three of the patients (1 2 and 3) and in the last three-quarters of the average curve in the CpK period approached the control values of the 1st, 2nd, 8th, and 14th weeks again showing that no major oedema was available for elimination

The 24-hour volume averaged 1 03 l for all patients in the Cp period versus 1 10 l in the CpK period this slight difference is significant (P = 0.035)

Sodium (fig 2) The 24-hour output always increased when cyclopenthiazide was administered but generally levelled off during the second week of medication (4th and 10th week) discontinuation of the drug always produced a compensating low output for about one week (7th and 13th week) There was no significant difference between the output in the Cp and CpK periods.

Chloride (fig 3) parallelled sodium when the output during the CpK period was corrected for the chloride in the tablets The Cp output of chloride was significantly higher than the corrected Cpk output In this connection it should be remembered that the value used for correction presupposes that all of the extra chloride in the tablets was excreted in the urine and that the urine was quantitatively collected If the urine were collected with an efficiency of say 0 9 instead of 1 0 the difference would no longer be significant

Potassium (fig 4) No significant change in the output could be detected in the 1 d and 2 d periods following Cp, but the first week of this period (3rd week) showed an excess output which was compensated for during the first week of C2 (7th week) The start of CpK caused a fall in the corrected values, and the corrected CpK output was significantly below the Cp output.

The discussion of the validity of comparing chloride output during Cp with corrected output during CpK also applies to potassium. Here, however the difference is still significant at a urine collection efficiency of 0.85 It is more likely that the considerably larger amount of potassium absorbed during the CpK period to a certain extent prevents depletion of the body potassium. The 13th week evinced no fall as compared with the 12th week this is in contrast to the result of a comparison between the 7th and 6th weeks, in which a pronounced fall was demonstrated.

There is an increased output of water sodium, chloride, and potassium during the first week after starting the treatment with cyclopenthiazide, either with or without supplementary potassium chloride, and a compensatory fall in the output after discontinuation of the diuretic. The greater part of the supplementary potassium chloride is excreted in the urine. When the chloride and potassium output values are corrected for the extra oral potassium chloride, no striking differences are found between the Cp and CpK periods. There is, however a slightly higher output of water after the addition of potassium chloride, and, of probably greater interest, no fall in the potassium output in the C3 period this might mean that no major depletion of the potassium pool occurred during treatment with cyclopenthiazide and potassium chloride.

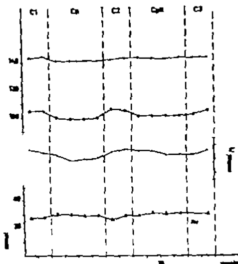


Fig 5. Weekly means for five patients except the value for total carbon dioxide in the 13th week which represents only one patient (3) who normally showed weekly averages between 24 and 27 mmol/l. The averages for the same patient in the 14th week are missing for all four serum determinations X—X serum concentration of sodium, O—O chloride, ●—● potassium, △—△ total carbon dioxide.

SERUM

Sodium. No significant changes were detected and the average curve of weekly means for the five patients fluctuated within 142 ± 2 mmol/l (fig 5)

Chloride (fig 5) In all patients a decrease was found when cyclopenthiazide was administered with or without additional potassium chloride (except in one patient (case 2) in whom the serum chloride remained virtually unchanged during the CpK period) The average fall reached 6 mmol/l in the Cp period and 4.5 mmol/l in the CpK period this difference, however is not significant.

Potassium. The individual variations in the weekly means are shown in fig 6 whereas the overall picture is demonstrated in fig 5 The serum potassium concentration of all patients fell during the

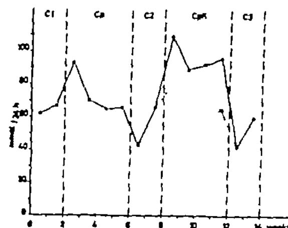


Fig 3 Urinary chloride 24-hour excretion. Weekly means for five patients. Squares represent values corrected for chloride added in tablets.

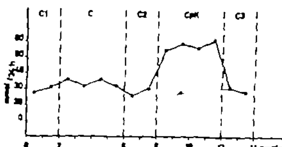


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URINE

The excretions of urine sodium chloride and potassium as well as the excretions of chloride and potassium corrected for the amount added in the tablets during the CpK period were calculated for each 24-hour period Comparisons were made for different lengths of time between the values before and after each shift of regimen e.g the means of chloride excretion for the last 24 48 and 168 hours of the Cp period were compared with the means of the corresponding first 24 48 and 168 hours of the C2 period Hereafter these periods of time will be designated 1 d, 2 d and 1 wk respectively Furthermore, the average 24-hour excretion in each of the 14 weeks was calculated

Volume The 1 d and 2 d comparisons for the values at the transition from period C1 — Cp and C2 → CpK showed only moderate variations, but mostly a rise, the 1 wk comparisons always showed an increase (fig 1) The changes at Cp → C2 and CpK → C3 were always a moderate decrease (except in one patient where a slight rise in the 2 d comparison was recorded) The weekly averages in the last three-quarters of the Cp period for three of the patients (1 2, and 5) and in the last three-quarters of the average curve in the CpK period approached the control values of the 1st, 2nd, 8th, and 14th weeks again showing that no major oedema was available for elimination

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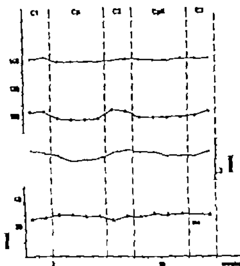


Fig 5. Weekly means for five patients except the value for total carbon dioxide in the 13th week which represents only one patient (3) who normally showed weekly averages between 24 and 27 mmol/l. The averages for the same patient in the 14th week are missing for all four serum determinations. × — × serum concentration of sodium, ⊙—⊙ chloride, ●—● potassium, △—△ total carbon dioxide

Serum

Sodium. No significant changes were detected and the average curve of weekly means for the five patients fluctuated within 142 ± 2 mmol/l (fig 5)

Chloride (fig 5) In all patients a decrease was found when cyclopenthiazide was administered with or without additional potassium chloride (except in one patient (case 2) in whom the serum chloride remained virtually unchanged during the CpK period) The average fall reached 6 mmol/l in the Cp period and 4.5 mmol/l in the CpK period this difference, however is not significant.

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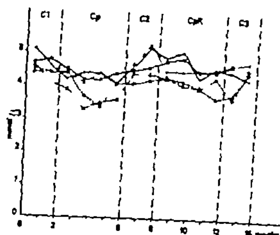


Fig 6 Individual weekly means of potassium in serum. ○—○ case 1 ●—● case 2, □—□ case 3 ×—× case 4 △—△ case 5

the Cp period and two patients (cases 1 and 5) reached values below 3.5 mmol/l. The mean values became normal during the C2 period The individual curves in the CpK period present a confusing picture. The average curve discloses a slight, but not significant fall which is significantly less marked than the Cp-decrease ($P < 0.001$) No values were below 3 7 mmol/l

Total carbon dioxide The average tendency was a slight significant elevation of total carbon dioxide (2 –3 mmol/l) during treatment with cyclopenthiazide, whether or not potassium was added (fig 5) No significant difference between the Cp and CpK periods could be detected.

The well known effects of benzothiadiazines viz. hypokalaemia hypochloraemia and baseosis, were encountered. The addition of 24—32 mmol potassium chloride to 0 75—1 00 mg of cyclopenthiazide daily caused a slightly (but not statistically significant) less marked fall in serum chloride, whereas the minor rise in total carbon dioxide remained virtually unchanged the fall in serum potassium was not seen in the average curve, but two patients showed a slow decrease during the four weeks (CpK)

Blood pressure

In one of the two normotensive patients (case 5) a very slight depression of the systolic pressure (5—10 mm Hg) was discernible, whereas the other one (case 4) showed no changes.

In the hypertensives the systolic levels in the Cp and CpK periods were about 20—25 mm Hg lower than in the control periods, and the diastolic pressure fell correspondingly by a maximum of 10 mm Hg

Miscellaneous results

The 24-hour urinary output of uric acid (fig 2) fell significantly during the first week of Cp ($P < 0.01$) but was again normal in the next week. No significant deviations were found when potassium chloride was added

The *serum uric acid concentrations* fluctuated seemingly without relation to the cyclopenthiazide treatment as did the total concentration of *leucocytes in the blood* and of *platelets in the plasma*.

Discussion

Of the five patients, four showed crural oedema as this did not diminish during treatment and as body weights varied only slightly the patients were considered to be in a fairly steady state in respect to water balance during the trial.

The biochemical values in the latter of the two weeks (C2 8th week) between the periods of medication (Cp and CpK) were essentially the same as in the pre treatment period (C1) In comparing the results of cyclopenthiazide treatment, without and with addition of potassium chloride, therefore, it was considered that the patients had been completely restituted before the beginning of the second course of diuretic.

One milligram (in one patient 0.75 mg) of *cyclopenthiazide daily for four weeks caused* the anticipated, rather slight rise in urinary volume, and this was compensated for when the drug was withdrawn. The changes in sodium and corrected chloride output were similar though more pronounced. The increased potassium output slowly returned towards normal during continued medication.

In accordance with Schafroth (12) and Sandoe and Olesen (10) no significant changes in the serum sodium concentration could be detected, whereas the chloride concentration fell by about 6 mmol/l and the concentration of total carbon dioxide rose slightly (by 2–3 mmol/l). The serum potassium concentration decreased consistently in two patients to 3.4 and 3.3 mmol/l, respectively. The values seemed to level off during the latter part of the treatment period, which may indicate that the rate of intracellular potassium loss was declining. Discontinuation of the diuretic promptly raised the potassium concentration in the serum. Schafroth (12) found no abnormal potassium concentration, but he used 0.25 mg cyclopenthiazide a day. Truniger and Siegenthaler (13) administering 0.50—2.0 mg, did not encounter hypokalaemia among ten patients with hypertension or refractory oedema. This is rather surprising, as Detli et al. (4) found an average fall of 0.7 mmol/l after three weeks of 0.5 or 1.0 mg cyclopenthiazide daily and Sandoe and Olesen (10) reported hypokalaemia in 39 % of oedematous patients on 1 mg cyclopenthiazide daily. We found two out of five patients to develop manifest hypokalaemia (i.e. < 3.6 mmol/l).

When 32 mmol (in one patient 24 mmol) *potassium chloride was added* to cyclopenthiazide, the daily output of urine became slightly higher than without potassium chloride. The increase in sodium output was unchanged. The corrected chloride output showed perhaps a slight decrease.

The output of potassium, when corrected for the extra intake, was lower than in the second halves of the control periods before and after (C2 8th week and C3 14th week). This is in keeping with the findings of Sandoe and Olesen (11) who found no change in potassium balance in five oedematous patients during seven days on one mg of cyclopenthiazide plus 40 mmol of potassium chloride.

The sodium concentration in the serum remained unaltered, but serum chloride fell, and not significantly less than without a supplement of chloride. The average decrease (4.5 mmol/l) is greater than the insignificant fall (1.5 mmol/l) found by Sandoe and Olesen (11) in 18 patients. The increase in total carbon dioxide was not influenced by the addition of potassium chloride — Sandoe and Olesen (11) reported a slightly lesser increase. The concentration of serum potassium showed individual variations, but no significant, average decrease, and no values below 3.7 mmol/l were encountered (cf. 11). The patients showed good tolerance of the coated tablets containing 600 mg potassium chloride per 0.25 mg cyclopenthiazide.

Conclusions

The older benzothiadiazines, when used for long term treatment, cause a complicating hypochloraemic, metabolic basosis and a negative potassium balance with hypokalaemia (5, 7, 14); the latest derivative, cyclopenthiazide (Navidrex®) is no exception when used in doses of 1 mg daily as shown in oedemat

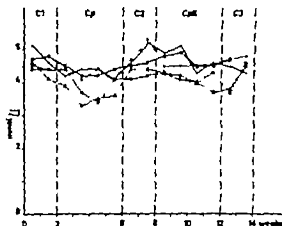


Fig 6. Individual weekly means of potassium in serum. ○—○ case 1 ●—● case 2, □—□ case 3, ×—× case 4 △—△ case 5

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rose during the two treatment periods. Cyclopenthiazide caused a fall in the concentration of potassium — in two patients to below 3.6 mmol/l the addition of potassium chloride resulted in a significantly less marked fall — never to below 3.7 mmol/l.

It is concluded that in patients with negligible excretion of oedema cyclopenthiazide causes the same, well-known alterations in electrolyte balance as other benzothiadiazines. Addition of 2,400 mg of potassium chloride to one mg cyclopenthiazide a day does not abolish the metabolic hypochloraemic basosis, but it reduces the loss of potassium, and, in our patients, prevented subnormal serum levels of potassium.

Acknowledgement

We wish to thank Jørgen Lorenzen, M.D. CIBA, Copenhagen, for his kind assistance and for a liberal supply of the drug in tablets under the registered trade names Navidrex® (containing 0.5 mg cyclopenthiazide) and Navidrex®/KCl (containing 0.25 mg cyclopenthiazide and 600 mg potassium chloride) and Mr. Nich F. Gyldeback, lecturer to The Royal Veterinary and Agricultural College, for the statistical analyses.

We acknowledge our indebtedness to the hospital nurses for the extra work involved by the daily collection of urine specimens and to the many laboratory technicians for a large number of careful analyses.

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ous patients (4 10) In our five patients with negligible excretory elimination of oedema, the same set of disturbances was elicited and subnormal serum potassium concentrations were encountered in two

Supplementary oral potassium chloride during treatment with benzothiadiazines is recommended by some authors (e.g 5 14) in order to counteract the tendency to cellular potassium loss. When using daily doses of 1 mg cyclopenthiazide, addition of 20—40 mmol potassium chloride is beneficial. This was shown in oedematous patients (11) and is apparent also from the present results in patients who showed no major elimination of oedema. Addition of 24—32 mmol potassium chloride does not prevent the hypochloraemia and baseosis.

Before long term treatment with one of the benzothiadiazines is instituted the hepatic and renal functions should be assessed. Liver cirrhosis additionally increases the excretion of potassium, and an impaired renal function tends to mask the baseosis and hypokalaemia. Apart from the special considerations required in such cases, additional potassium chloride in amounts of 20—40 mmol daily should be given (with cyclopenthiazide 0 5—1 0 mg) A regular control (initially every two weeks) of the serum concentrations of potassium and preferably also of standard bicarbonate (total carbon dioxide) chloride, and sodium is advisable, as the patients may differ in their reactions, so that the dosage has to be individualized If used as the sole guide, the serum level of potassium may give unreliable information because a considerable, negative potassium balance may develop despite an only moderate decrease in the serum level.

Aldosterone inhibitors may somewhat correct the changes produced by benzothiadiazines, and furthermore, may increase the output of water in oedematous patients (8) Therefore, a suitable combination of cyclopenthiazide and spironolactone might be of value in some cases.

When excretory elimination of oedema is the sole therapeutic goal the administration of a benzothiadiazine for only three consecutive days a week may yield the desired effect without harmful changes in the electrolytes (9) when an antihypertonic effect is desirable, however continued daily dosage is necessary

Summary

One of the benzothiadiazines, cyclopenthiazide (Navidrex®) was administered to five patients without demonstrable liver disease and without oedema that could be appreciably influenced during the trial. The trial consisted of three control periods of two weeks each, which preceded and followed two four week periods of treatment with one milligram of the drug daily in the latter treatment period a daily supplement of 2,400 mg (32 2 mmol) potassium chloride was given

During the first week of each treatment period the urinary output of water sodium, chloride, and potassium were increased and a compensatory retention occurred in the first week of the following control periods. When corrected for the potassium chloride added to the tablets, the output of potassium was significantly lesser during the treatment period with a supplement of potassium chloride than during the corresponding period without this supplement, and no fall in the output occurred during the final control period

The chloride concentration in the serum fell and that of total carbon dioxide

rose during the two treatment periods. Cyclopenthiazide caused a fall in the concentration of potassium — in two patients to below 3.6 mmol/l the addition of potassium chloride resulted in a significantly less marked fall — never to below 3.7 mmol/l.

It is concluded that in patients with negligible excretion of oedema cyclopenthiazide causes the same, well-known alterations in electrolyte balance as other benzothiadiazines. Addition of 2,400 mg of potassium chloride to one mg cyclopenthiazide a day does not abolish the metabolic hypochloraemic basosis, but it reduces the loss of potassium, and, in our patients, prevented subnormal serum levels of potassium.

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the Department of I ternal Medicine (Head E. Roelsen, M. D) the Central Hospital, Silkeborg, Denmark

Polyneuritis after Nitrofurantoin (Furadantin®) Therapy

A Survey and Report of Two New Cases

By

E. Roelsen

Nitrofurantoin was introduced in the treatment of urinary-tract infections about 10 years ago and has since been widely used. The drug seems especially to be effective against infections by Escherichia coli. In 1958, Knud O Møller (23) wrote in his textbook of pharmacology that "the drug is only slightly toxic. Now and then nausea and vomiting may occur Apart from occasional idiosyncratic reactions, no other side effects re seen — However it must be remembered that the drug has been used only for few years

Almost simultaneously authors from Denmark nd Sweden reported the occurrence of neurotoxic side effects in the form of peripheral polyneuritis manifested by distal paraesthesiae, dysaesthesiae hypaesthesiae muscular wasting diminished reflexes and muscular weakness of the hands and feet. Larsen and Bertelsen 20) and Olivarius (26) reported one ase each Falck et al (12) four and Hafström (14) fiv cases. In the very severe case described by Briand nd Tygstrup (4) there were severe histological changes in the peripheral nerves, manifested by fragmentation and demyelinisation of the nerve sheaths, and in the motor cells of the anterior horns, especially in the cervical and lumbar parts of the spinal cord Circumscribed areas of atrophic muscle fibres were also demonstrated.

A recent convincing study by Collings (6) deserves special mention. He described three cases of polyneuritis after Furadantin therapy and one after administration of a closely related agent, nitrofurazone (Furacin®) in the treatment of a metastasising carcinoma of the testis. Like the Scandinavian authors, Collings emphasised that it is important to avoid long-continued therapy with these drugs, especially if renal function is impaired. Another American case reported by Uesu (33) is also in support of this view The significance of these measures have recently been further emphasised by English authors reporting 10 cases (9 18 21 32)

According to information received from the licensers, Eaton Laboratories, Norwich, New York, the Danish phar-

Submitted for publication July 17 1963.

maceutical factory Pharmacia (30) stated in 1962 that up to that time presumably 17 million "courses of treatment with Furadantin had been given in the U S A alone but only seven cases of polyneuritis attributable to the drug had been published in the U S A., while the factory had been informed of the occurrence of 14 additional cases by personal communications, viz. eleven from the U S A and three from Australia.

By a review of the literature available to me I have traced a total of 31 published cases of polyneuritis after Furadantin therapy Reported cases with paraesthesiae suspected to be due to Furadantin (15 27) are included in these 31 cases. This case material together with two personally observed cases forms the basis of the present paper[1]

Palmlöv and Tunevall (27) summarised all the side effects observed in 440 Furadantin treated patients as follows nausea 10 % vomiting 1 % skin rash 1 % headache 1 % rheumatic pain in one patient fever in two and paraesthesiae in two patients (see further below) The commonest side effect seems to be nausea which is due to a direct action on the gastric mucosa and may be avoided by the use of coated tablets (25)

A few additional side effects are reported in the literature leukocytopenia (15) which may however be queried a few cases of haemolytic anaemia in Negroes and certain groups of the population in Mediterranean countries and the Near East (7 34) an isolated case of megaloblastic anaemia (2) one case of allergic jaundice (11) and one of recurrent allergic pleuro-pneumonia (17) For the sake of completeness, it should be mentioned that a closely related substance, furaltadone (Altafur®) which has been used during recent years in the U S A. and Australia as an antibacterial agent against penicillin resistant staphylococci, has given rise to paralysis of cerebral nerves in one case (3) and to atrial fibrillation in four (31)

There is a general trend to apply continuous or at least, frequent intermittent bacteriostatic treatment to urinary-tract infections (24) In Scandinavia, attention is at present focused on long term treatment of pyelonephritis with sulphonamides or nitrofurantoin (5 10 19 35) It is therefore reasonable, on the basis of two cases observed in our department to emphasise the potential risk involved in long-continued Furadantin therapy This risk has also recently been pointed out by Effersoe (8) In this connexion it is only fair to mention that, in our hospital this drug has not infrequently been very helpful in the treatment of urinary tract infections, and that, when given for short periods in the doses recommended by Pharmacia (29) it will scarcely involve any serious risk in the majority of cases. On the other hand if long term therapy be used it seems necessary to take certain precautions (see below)

Case reports

Case 1 A married former labourer aged 76, was admitted to the Department of Internal Medicine on Sept. 6 1962 His past history was essentially that of good health, without any evidence of alcohol addiction or lead poisoning In 1959 he had prostatic hypertrophy which was treated in the Department of Surgery with an indwelling catheter and,

[1] It should be added that Pharmacia by kind permission of the licensers has allowed me to go through the records of unpublished cases of Furadantin polyneuritis filed at the Eaton Laboratories. These cases do not differ from those reported in the literature.

3½ months later with suprapubic prostatectomy. Serum creatinine 0.9–1.1 mg/100 ml. At that time, atrial fibrillation and slight hypertension were present.

In May 1959, treatment with Furadantin, 100 mg three times daily was started by his physician because of cystitis, and this treatment was continued for more than 3 years. A total dose of 363 g Furadantin was given. The patient stated that when on occasion he stopped taking the drug the urine immediately became cloudy. His prescriptions were mostly renewed by application to the doctor over the telephone.

About 12 months before the admission to this department, after total dose of about 250 g Furadantin, the patient began to suffer from numbness and tingling of the feet and, little later of the hands. During the last 4 or 5 months, he had, in addition to Furadantin, also been treated with Ilidar® tablets, because the doctor believed that the conspicuous motor and sensory disturbances, especially wasting of the muscles of the hands and feet, were due to arteriosclerotic phenomena. Gradually the muscular power of the arms and legs and especially of the hands and feet became greatly impaired. Eventually the patient could not eat or shave unaided, and the gait became increasingly disturbed.

Physical examination revealed, apart from slight hypertension (B.P 190/110 mm Hg) and slow perpetual arrhythmia, non-sclerotic well-preserved man with pronounced symmetrical polyneuritis and distinct muscular wasting of the upper limbs *increasing distally* on the forearms and, particularly in the interosseous muscles of the hand, and also of the lower limbs, also here particularly marked peripherally. The muscular power was greatly reduced. Distally increasing hypaesthesia and hypalgesia were also noted. The hands assumed typical drop-wrist position, and the fingers were flexed in claw position. The extension of the wrists was nearly and that of the interphalangeal joints of the fingers completely abolished the abduction of the thumbs was markedly impaired. The feet assumed characteristic drop-foot position, with abolition of dorsiflexion, pronation and supination. The reflexes of the upper limbs were normal, the patellar reflexes weak, and the Achilles-tendon and plantar reflexes absent. The sensation of deep heel pain was preserved vibratory



Fig. 1. Case 1. Bilateral paralysis of the radial nerve.



Fig. 2. Case 1. Bilateral paralysis of the ulnar and median nerves.



Fig. 3. Case 1. Bilateral paralysis of the peroneal nerve.

sensation was normal. The muscular tonus of the arms and thighs was normal. There were no disturbances in co-ordination, and the position sensation was normal.

The hands were cool but the pulsation of the radial arteries was satisfactory The feet were somewhat cyanotic. The pulsation was good in the femoral and popliteal arteries, but could not be felt in the arteries of the feet. The condition on admission is illustrated in figs. 1—3

Special investigations Height 176 cm weight 74.5 kg Body temperature normal. Hb. 94 E.S.R 47 mm/hour Differential count of white blood cells normal. The Wassermann, Kahn and gono reactions were negative. Lumbar puncture showed no cells. Total protein 54 mg/100 ml The urine contained protein (0 1 %) and microscopy revealed Gram-negative rods and Gram-positive cocci, which were resistant to Furadantin. Moderate pyuria. Serum creatinine 1 2 mg/100 ml. Serum electrolytes normal.

Muscle biopsy showed atrophic changes in the muscle fibres compatible with a neurogenic pathogenesis (Dr K. Aa. Lorentzen)

Oscillometry showed normal conditions.

Ophthalmological examination revealed hypertensive changes in the eyegrounds (grade I)

Admission to the Department of Neurology Aarhus Kommunehospital (Sept. 26—Oct. 4 1962) Clinical examination here revealed the same findings as stated above. Electromyography of the right and the left m. extensor dig comm. showed no loss of motor units. Denervation potentials were observed in the left m. extensor dig comm. The observed duration of the potentials in the right extensor dig comm. was 13 4 m/sec (normal 11 7) The right and left m. tibialis anterior showed pronounced loss of motor units and no signs of spontaneous activity The findings were suggestive of neurogenic affection.

Treatment. The polyneuritis was treated with vitamin-B preparations (B-combin® Betolvex® (cycobemin) pyridoxine and electrostimulation by Myotensor" After 7 weeks, the patient could almost extend his fingers, while the improvement in the feet was quite unsatisfactory The gait was still disturbed, of the characteristic steppage type. The condition is unchanged 9 months later

Case 2 A married female dressmaker aged 55 was admitted on June 20 1962 Since 1956, she had had three attacks of "pyelitis." Since the menopause 5 years previously she had often taken Coderphen® and Codaron® tablets because of headache. On May 16, 1962, the patient was admitted to the Department of Surgery with right-sided lumbar pain for observation for pyonephrosis, which, however was not present. Serum creatinine 2.8—3.2 mg/100 ml blood urea 225 mg/100 ml. The urine contained protein (0 1 %) Pyuria and bacteriuria were present, mainly Gram-negative rods. She was treated with chloramphenicol and Reverin® and then transferred to the Department of Internal Medicine. Height 162 cm weight 56 kg Blood pressure 145/115 mm Hg. The patient was treated with Furadantin for four periods. Sensitivity tests usually showed that the organisms were sensitive to this drug and to chloramphenicol. However on principle, we never use the latter agent in urinary infection. The duration of the periods was 11 9 5 and 3 days, with total doses of 4 4 3 6, 2.0 and 1.2 g respectively The intervals between the periods were 3, 24 and 33 days.

After the second period, the patient became nervous and complained of numbness and tingling in the fingers and feet. These symptoms increased in intensity during the next two months. There gradually developed impaired sensibility in the distal parts of the hands and feet, ending in wasting of the interosseous muscles of the hands and slight drop-foot on the left side, accompanied by impaired gait. At first we were unable to interpret these phenomena, which seemed to be of a functional nature. In a special psychiatrical report it was stated that "a polyneuritis exists, probably with a hysterical superstructure, since the sensory disturbances have a circular border

Additional treatment. Streptomycin and sulphamethizole were given for shorter periods and Gesalol® for a longer period. Moderate doses of sodium bicarbonate were also given. The polyneuritis was treated in the same way as in case 1 Four months later the neurological symptoms had almost disappeared. The condition was otherwise marked by fatigue and anaemia. At intervals of varying length, the patient was seriously ill, presumably due to renal papillary necrosis. The renal function was invariably greatly impaired serum creatinine about 4 mg/100 ml, but the serum electrolytes including calcium and phosphate were maintained within normal limits.

Comments

For several years, we have administered Furadantin only for short periods because of the neurotoxic properties of the drug However only when we had had the opportunity to observe the above-mentioned grotesque case 1 did we appreciate the characteristic manifestations of mild polyneuritis which were presented in the early phase by our case 2, i. e. slight paraesthesiae which were suggestive of functional impairment. A study of the literature revealed that as early as 1954 Hasen and Moore (15) in their description of the side effects observed in 100 nitrofurantoin-treated patients, mentioned two patients with paraesthesiae which developed one week after the institution of the therapy subsided when the drug was withdrawn and re-appeared in one of the patients when the administration was resumed. The authors stated that the reactions might be due to some form of neuritis. In 1956 Palmlöv and Tanevall (27) made the same observation. These two observations are of essential importance in establishing Furadantin as an aetiological factor in polyneuritis. In the cases of polyneuritis subsequently reported in patients receiving Furadantin there is every probability that the disease was referable to the drug partly because there is no other explanation, and partly because improvement may occur after the withdrawal of the drug However it should be noted that Asbury (1) has recently pointed out that similar cases of polyneuritis may occur as complication of uraemic conditions in which Furadantin has not been given, but in which dialysable metabolites may presumably be incriminated. It seems that such cases are extremely rare. Polyneuritis occurring in association with administration of Furadantin should always be regarded as being caused by this drug

Collings (6) writes that presumably the urologist may well fail to pay attention to complaints of paraesthesiae, and that cases of this neuritis may be more frequent than so far assumed. Recently Martin et al., (22) who observed a case in one of their *colleagues*, called attention to the fact that the occurrence of paraesthesiae during Furadantin therapy is an important warning indicating that the therapy should be discontinued. However in a few cases, the paraesthesiae did not develop until after the withdrawal of the drug

In the vast majority of cases, polyneuritis attributable to Furadantin developed in patients with impaired renal function (possibly predisposed by metabolic disturbances as mentioned above (??)) and after prolonged treatment. Loughridge (21) reported that the plasma level of nitrofurantoin in patients with normal renal function receiving 500 mg daily was 1.8 μg/ml. In a patient with severe polyneuritis and greatly impaired renal function (blood urea 300 mg/100 ml, total plasma CO 12 mEq/1) she found levels ranging from 5 1 to 6.5 μg/ml. As regards the duration of the treatment before the onset of the initial symptoms of polyneuritis, it may be stated that one case has been reported (13) in which treatment for only 10 days with a total dose of 5 g ga e rise to prolonged disabling polyneuritis in a patient with moderately decreased glomerular filtration, but in whom co-existing nephrosis was present. It is known that elimination of the drug involves both glomerular filtration and tubular secretion (21) Of our two cases one represents the grotesque extreme after a total dose of 563 g renal function

Table 1 Survey of 21 previously reported cases

Author	Year	No. of cases	Duration of therapy	Total dose (g)	Estimated severity of polyneuritis	Estimated renal insufficiency	Disease
Hasen & Moore (15)	1954	2	16 days 7 days	9.6 2.7 (?)	(+)	(?) Normal	Pyelonephritis
Larsen & Bertelsen (20)	1956	1	39 days	15.6	++	(+)	Cystitis, carcinoma of the bladder
Olivarius (26)	1956	1	2 courses, 9 and 16 days	10.7	+++	+++	Recurrent pyelonephritis
Hafström (13)	1957	1	14 days	4.2	+++	+(+)	Cystitis, nephrosis
Briand & Tygstrup (4)	1959	1	6 months, intermittent	37	+++	+++	Pyelonephritis, necrotising papillitis, nephrolithiasis
Collings (6)	1960	3	120 days 59 days 35 days	48 24 14	+++ ++ ++	?	Cystitis, two in prostatic enlargement
Ueru (33)	1962	1	100 days	40	+++	+(?)	'Chronic renal infection (arteriosclerotic heart failure, anaemia)
Loughridge (21)	1962	1	6 weeks	13.8	+++	++++	Pyelonephritis, paraplegia
Ellis (9)	1962	6 (a)	Repeated courses, 13 months	71	+++	+	Urinary infection, prostatic enlargement
		(b)	3 months	17.1	+++	+++	Urinary infection, carcinoma of prostate
		(c)	11 weeks	34.4	+++	+	Urinary infection, urethral stricture
		(d)	4 weeks	16	++	++	Urinary infection, prostatic enlargement
		(e)	2 courses, (4+1) weeks	14.8	+++	++	Pyelonephritis
		(f)	5 months	38.8	++	++	Urinary infection, neurogenic bladder
Martin et al (22)	1962	1	3 courses, every other week for 5 weeks	pprox 8.4	(+)	Normal	Recurrent urinary infection, prostatitis
Spencer (32)	1962	2	9 months 9 months	163 82	+++ +++	++ ++++	( ) Ileo-cystoplasty pyelonephritis (b) Pyelonephritis
Jordan (18)	1962	1	14 weeks	50	+++	Normal	Cystitis, prostatic carcinoma

being normal, and the other a mild to moderate affection after a total dose of 11.2 g given in four courses, renal function being greatly impaired.

For the purpose of comparison, table I gives a survey of the duration of treatment, total doses of Furadantin the severity of polyneuritis and renal insufficiency and the basic disease in 21 previously reported cases in which, on the bases of the data stated, fairly accurately assessment of these factors has been possible.

It should be noted that in several of the cases with large total doses, the polyneuritis was ushered in by paraesthesiae at a time when the total dose was considerably smaller just as in our case 1 Not infrequently the diagnosis was made only when the polyneuritis was fully developed. On the whole it must be admitted that the dosage in all published cases, including our own, was generally appreciably larger than that recommended by Pharmacia (29)

In our first patient, who had normal renal function, the polyneuritis developed after treatment for ? years. The drug is reported to be eliminated very rapidly and, as mentioned, the blood concentration is low but in spite of this minimal concentration the drug is capable of producing the characteristic nerve lesions in long-continued treatment.

The polyneuritis is due to a prolonged toxic influence on the nerve cells, possibly through interference with the carbohydrate metabolism of these cells. Paul et al (28) demonstrated that nitrofurantoin and allied substances interfere with the anaerobic formation of acetyl coenzyme A from pyruvate and co-enzyme A. Their experiments were performed with testicular tissue, but the results may also apply to nerve cells. Certain features are suggestive of the development of allergy In several cases, it seems as if the side effects and polyneuritis after Furadantin treatment have aggravated, or developed, in a second course of administration (see Falck et al. (12) (case 1) Ellis (9) (case 5) and the case reported by Olivarius (26) The reactions in our case 2 are also suggestive of this possibility It was briefly mentioned in the beginning of this paper that Furadantin has also occasionally induced allergy in some other respects.

It is understandable that Furadantin polyneuritis mainly occurs in the older age groups in which urinary-tract affections requiring treatment are most common. Our patients aged 55 and 76 represent the usual age group but the condition has been encountered in patients whose ages ranged from 35 to 85 years. It is equally frequent in both sexes (7)

The peripheral nerve lesions which, in turn, lead to the characteristic muscular changes consist, as already mentioned in Wallerian degeneration of the myelin sheaths. Briand and Tygstrup's demonstration (4) of this was confirmed by Loughridge (21) and Ellis (9) Collings (6) made the same observation in a Furacin polyneuritis. There are no inflammatory reactions. The condition consists of changes in the peripheral nerves and the second motor neurone attributable to a toxic or possibly allergic influence, and presumably also of changes in the spinal ganglia, although the latter have not been mentioned in the two cases subjected to autopsy Many authors speak instead of polyneuritis of polyneuropathy In our case 1 we confined ourselves to demonstrating the characteristic histological muscle changes, since we found that a nerve-tissue biopsy would

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be too hazardous in this grossly disabled patient.

Obviously the prognosis depends on the severity of the polyneuritis. If it is as severe as seen in our case 1 the condition is usually irreversible.

Discussion and conclusions

In view of the circumstances the number of cases of polyneuritis attributable to Furadantin so far published is almost incredibly small. Some mild cases must be supposed to have passed unnoticed and some severer cases may either have escaped recognition or have not been published. I am aware that Pharmacia (30) knows of some such cases in Denmark, and personally I also know of one case seen in another medical department. Very severe cases like our case 1 are rarely allowed to develop because attention is generally paid to the warnings voiced in the reports and by the manufacturers. Considering the common use of Furadantin and the relatively small number of published cases of polyneuritis caused by this agent which must, however be interpreted as minimum figures, it is presumably justified to regard it as fairly non toxic. In view of the experience gained, with side effects in the form of polyneuritis, which may sometimes be disabling it must nevertheless be recommended to avoid long-continued treatment and to be very cautious in the presence of impaired renal function. Here it is appropriate to recapitulate the dosage schedule published by Pharmacia (29) "Acute mild urinary tract infections 40 mg four times daily for 5—7 days acute severe or chronic infections 100 mg three or four times daily for 5—7 days. Maintenance dose in chronic cases 20 mg four times daily. If this schedule is followed the total doses in the first two instances will be 1 12 and 2.8 g, respectively As compared with those cited above, which resulted in polyneuritis, these total doses are very modest. In this connexion it is worth noticing that Hardem and Muri (16) obtained good clinical results on a dosage of 25 mg six times daily for 6—7 days. Bucht et al. (5) recommended a still lower dosage adjusted to renal function 50 mg three times daily to patients with relatively well preserved renal function i. e. with a serum creatinine level below 2 mg/100 ml. In cases with serum creatinine ranging from 2 to 4 mg/100 ml, they used 50 mg given twice daily and in patients with a serum creatinine level above 4 mg/100 ml only 25—50 mg in 24 hours. Judging from the report of these authors, this low dosage used for a prolonged period in combination with other chemotherapeutic agents, seems to contribute materially to the sterilisation of the urine in several cases. By this dosage schedule it may be possible to avoid the development of polyneuritis, but strict attention must be paid to the occurrence of paraesthesiae.

Lastly consideration may be given to the significance of Furadantin in the effective elimination of deep-seated parenchymatos inflammatory changes. In view of the minimal blood concentrations that can be achieved it is doubtful if this drug can be regarded as more than an adjunct to the far more effective sulphonamides and usual antibiotics in the treatment of chronic pyelonephritis and urinary tract affections. In spite of this it is a general clinical experience that Furadantin has often periodically been very helpful as a bacteriostatic agent in the treatment of chronic urinary tract infections, especially when resistance to

otherwise effective sulphonamides and antibiotics develops. This effect must be ascribed to the fairly high concentrations in which the drug is excreted in the urine. If the renal function is greatly impaired, it is, according to Loughridge, unlikely that an adequate urinary concentration can be achieved to exert an effective inhibition of bacterial growth. This fact also weighs against the use of the drug in the presence of grossly impaired renal function.

Summary

1 A brief survey is given of the side effects, especially polyneuritis, occurring after Furadantin therapy in urinary tract infections. With the two patients described in the present paper a total of 39 cases of Furadantin-induced polyneuritis are on record. The Eaton Laboratories, Norwich, N. Y. know of 14 additional cases reported to them by personal communications. Pharmacia, Denmark, and the author are similarly aware of a few other unpublished cases.

The polyneuritis is attributable to a toxic or possibly allergic effect of the drug, with degeneration of the myelin sheaths developing after long-continued treatment, and especially in patients with impaired renal function, in whom the injury may be of rapid onset. The manifestations consist of slight distal sensory disturbances which gradually increase in severity abolished reflexes and loss of motor units followed by pronounced muscular wasting and ending in a completely disabling condition.

2 Two cases of typical polyneuritis after Furadantin therapy are described.

A man, aged 76 with cystitis arisen after prostatectomy but with normal renal function received a total dose of 363 g Furadantin during a period of 3½ years. After the lapse of 2½ years typical severe symmetrical polyneuritis with paralysis of the radial ulnar median and peroneal nerves developed. Typical hypaesthesia was present and pronounced muscular wasting in the distal parts of the limbs developed. Electromyographic studies showed delayed conduction in the muscles of the forearms and completely abolished conduction in the muscles of the lower legs. A muscle-biopsy specimen showed degenerative changes as in neurogenic affection. Treatment with vitamin B preparations and electrostimulation therapy ("Myotensor") for 9 months resulted in some improvement in the condition, especially as far as the hands were concerned, but the gait remained greatly impaired. The condition must be regarded as grossly disabling.

In a woman, aged 55 with pyelonephritis ("tablet kidneys") and greatly impaired renal function moderately severe polyneuritis developed after four short periods with Furadantin therapy (total dose 11.2 g) Treatment with vitamin B preparations and electrostimulation ("Myotensor") for several months resulted in considerable improvement in the condition.

3 The author warns against prolonged Furadantin therapy Caution should be exercised in administering the drug to patients with impaired renal function in the presence of severe renal insufficiency Furadantin should not be attempted at all. Dosage schedules used by Norwegian and Swedish investigators are mentioned these are somewhat lower than that stated by the licensee (Pharmacia, Denmark) and seem to be less hazardous.

4 In view of the very common use of Furadantin in the treatment of urinary-tract infections and the relatively small

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Obviously the prognosis depends on the severity of the polyneuritis. If it is as severe as seen in our case 1 the condition is usually irreversible.

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the Second Department of Pathology and the First Medical Service Sahlgren's Hospital, Göteborg, Sweden

The Renal Concentrating Capacity in Albino Rats after Long-term Consumption of Phenacetin, NAPA (N-acetyl-p-aminophenol) and Acetylsalicylic Acid

By

Lennart Angervall, Leif Lehmann and Ulla Bengtsson

During recent years several investigators have presented clinical data indicating a nephrotoxic effect from phenacetin (3 7 8, 9 11 12, 14) There are also animal experiments suggesting such an effect (1 2, 10, 15) The animal studies are mainly morphologic examinations of the kidneys after feeding phenacetin. No renal function study in animals fed phenacetin is known

This paper deals with the renal concentrating capacity in rats after long-term consumption of large amounts of phenacetin and NAPA (N-acetyl-p-aminophenol) — a metabolite of phenacetin — and of acetylsalicylic acid. Quantitative estimates of the urinary sediments were also performed.

Material and methods

Seventy-eight female rats of Wistar strain were used. All the rats received a pulverized standard food as described in an earlier study (2) The rats were divided into four groups according to the drugs added to the food. The groups are given in table I

Submitted for publication July 22, 1963.

The approximate drug intake calculated on the mean weekly food intake and the mean body weight, in the various groups are also given in table I. Equimolar amounts of phenacetin and NAPA were given. The rats in the phenacetin group received approximately 0.4 g phenacetin daily per kg body weight. The intake of NAPA was similar and the intake of acetylsalicylic acid was 0.25 g daily per kg body weight.

The rats were allowed to eat and to drink tap water ad libitum. The period of this regimen comprised 41—42 weeks for all rats. The food intake was determined per week for each group, and each rat was weighed once a week.

The renal concentrating capacity was estimated in all rats, during a period of 10 days, at the end of feeding the drug, as rule in 2 rats from each group per day The following procedure was used water was withdrawn for 18 hours, from 9 p.m. until 3 p.m. the following day

During this test the rats were kept in individual cages with a fine-meshed bottom from which the faeces could easily be removed. Below the grid there was funnel discharging the urine into flask with narrow orifice.

The osmolality was determined in urine collected from 9 a.m. till 3 p.m., i. the last

number of published cases of polyneuritis after this therapy, it must be assumed that the above mentioned rules have largely been adhered to However in our experience, it is likely that some mild to moderately severe cases have passed unnoticed.

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The osmolality was determined in urine collected from 9 a.m. till 3 p.m., i.e. the last

Table I Classification of experimental rats and drug doses

Group	Drug added to food	g drug/ kg food	g drug/ kg body weight/ day	No of rats
I	None	—	—	20
II	Phenacetin	5.35	0.45	18
III	NAPA	4.55	0.40	22
IV	Acetyl-salicylic acid	2.70	0.25	18

Table II The renal concentrating capacity in rats after 41—42 weeks of drug intake

Group	Drug	Maximal urine osmolality mOsm/kg H_2O	t
I	None	2734±72	
II	Phenacetin	2206±97	4.39
III	NAPA	2361±71	3.69
IV	Acetylsalicylic acid	2594±121 (2671±90)	1.00 0.45

Mean osmolality after exclusion of two pyuric rats.

6 hours of water withdrawal. The osmolality was calculated from the freezing point depression measured with a thermistor and a resistance bridge.

The urine collected from 9 p.m. till 9 a.m. (the first 12 hours of water withdrawal) was used for a quantitative estimate of the urinary sediment. Red and white cells were counted in a Burker cell-count chamber.

After the performance of the function study during the 42nd week, all rats were inoculated with Escherichia coli. 0.5 ml of a 6-hour culture was injected into vena jugularis, a dose reckoned to contain approximately 500 mill bacteria. Eleven or twelve days later nephrectomy of the left kidney was performed. The aim was to feed the same drugs for another 6

Table III Quantitative counts of blood cells in the urine per 12 hours

Group	Drug	Erythrocytes (Mean values)	Leucocytes
I	None	2111±548	683±279
II	Phenacetin	23333±114711 1428±453[1]	822±300
III	NAPA	1772±505	1409±516
IV	Acetyl-salicyl acid	2150±250	5800±3340 666±510[2]

[1]Mean value after exclusion of 4 haematuric rats.
[2]Mean value after exclusion of 2 pyuric rats.

weeks. However the majority of the rats in all four groups died in pneumonia, and thus a comparative study of the degree and frequency of interstitial nephritis could not be made adequately for either left or right kidneys.

Results

Dietary intake curves have been plotted in fig 1 Body weight curves are shown in fig 2 There are no significant deviations among the four groups Only during the last weeks was there a drop in the food intake in the NAPA-group, but there was no corresponding drop in the weight curve.

The renal concentrating capacity measured as urine osmolality is shown in table II

The mean osmolality of the phenacetin group (2206) is significantly lowered in comparison with the control group (2734) $P < 0.001$ The osmolality of the NAPA group (2361) is also significantly lowered, $P < 0.001$ In the acetylsalicylic acid group two values are given the mean value for the whole group (2594) and in parentheses the mean value for 18 rats (2671) after exclusion of two rats with a massive pyuria. These two rats had an

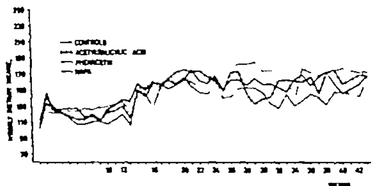


Fig. 1. Dietary intake curves.

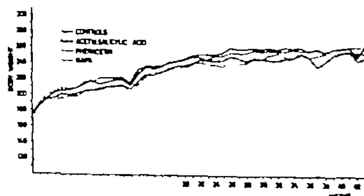


Fig. 2. Body weight curves.

osmolality which was markedly lower — 1890 — than for the rest of the group. It is probable that these rats had interstitial nephritis at the time of the concentrating test. Neither of the two mean values for osmolality in the acetylsalicylic acid group differed significantly from the control group value.

The urinary blood cell counts are shown in table III. The mean count of erythrocytes in the control group was 2111 and that of leucocytes was 888 per 12-hour night urine. The corresponding counts were similar in the other three groups except for 4 rats in the phenacetin group and 2 rats in the acetylsalicylic acid groups. The four phenacetin rats had high erythrocyte counts, 50 000—150,000 and the two acetylsalicylic acid rats had pyuria, 52,000.

Discussion

The rats in the phenacetin and NAPA groups received approximately 0.4 g phenacetin and NAPA daily per kg body weight. A corresponding intake in man would amount to 25—30 g a day — an excessive dosage. The admixture of phenacetin or NAPA to the food did not effect the dietary intake or inhibit the body growth.

The rats in the acetylsalicylic group received approximately 0.25 g acetyl salicylic acid daily per kg body weight. This dosage was estimated to be the highest dose without general toxic effects. In an earlier experiment the intended dose was 0.5 g/kg body weight, but the rats developed hyperphagia, lost weight, got massive intestinal bleedings and died prematurely (1). In the present study the acetylsalicylic group was similar in dietary intake and weight curve to the other groups.

Clinically a relationship exists between phenacetin abuse and renal papillary necrosis (3, 6, 7, 8, 9, 11, 12, 14, 15). Therefore, it is probable that the concentrating capacity is one of the renal tubular functions that will be affected earliest during phenacetin consumption. Hence the use of the concentrating test in this study.

The study has shown that long term feeding of phenacetin significantly decreased the concentrating capacity. The result is in agreement with the above mentioned assumption.

NAPA (N-acetyl p-aminophenol) has in recent years been introduced in analgesic compounds. As NAPA is a metabolite of phenacetin it is reasonable to suspect that NAPA should also have a nephrotoxic effect. A few experimental studies have been made suggesting that NAPA causes lesions similar to those from phenacetin (1, 2, 5).

The present study supports the suspicion that NAPA is a nephrotoxic agent as it caused a significant decrease of the concentrating capacity.

Salicylates are the most common of all analgesics and are usually assumed in the clinic to be non toxic when given in therapeutic doses. In a clinical material of chronic pyelonephritis with a high frequency of renal papillary necrosis, excessive use of phenacetin was admitted by 60 per cent of the patients, but there were no patients who had taken salicylates daily for years (3). Nor has this study pointed to a nephrotoxic effect from salicylates, the acetylsalicylic acid group not differing significantly from the control group.

It has been discussed whether phenacetin alone can produce morphologic renal damage or if the effect of phenacetin is just to lower the renal resistance against infections (3, 6, 13). In the present study it may be of interest that no difference was demonstrated histologically between non inflammatory renal tissue in the control group and in the three drug groups.

The present quantitative estimates of the urinary sediments showed no difference between the drug groups and the control group except for two rats in the acetylsalicylic acid group with a massive pyuria and four rats in the phenacetin group with a microscopic hematuria. The results suggest that no gross urinary tract infection was present at the time of the function tests, and seem to exclude infection as a possible cause of the difference in concentrating capacity between the phenacetin and NAPA groups on the one hand and the control group on the other.

A functional impairment might well precede the morphologic damage of the kidneys produced after long term phenacetin consumption. If the animals had been sacrified immediately after the concentrating test further light might have been shed on this question. The study was planned to investigate the question of increased susceptibility to bacteria, but this part of the investigation went astray probably because of too high amounts of injected bacteria.

The significance of haematuria in four phenacetin rats is unclear. Haematuria is a common feature in renal papillary necrosis in man, and three of the four rats had papillary changes when they were autopsied subsequent to injection of bacteria. However most of the rats, even in the control group, had advanced interstitial and papillary changes of the kidneys at that time.

Two rats in the acetylsalicylic group had a pyuria and a markedly lowered concentrating capacity and were considered as pyelonephritic at the time of the concentrating test. It is not known whether the rats were normal at the beginning of the drug intake, as no checks of renal function and urinary sediments were performed at that time. The other rats in this group did not differ from the control group. Clausen and Harvald (4 a) found in short term studies in man that salicylates provoked a greater increase of leucocytes and especially of erythrocytes in the urinary sediment than did phenacetin. Clausen (4 b) found that rabbits, inoculated with *E.coli*, developed inflammatory kidney changes if they were pretreated with acetylsalicylic acid. These changes were similar — though less marked — to those after pre-treatment with phenacetin. The present study provides no evidence of an action of acetylsalicylic acid on rat kidney either specific or non-specific.

Summary

The renal concentrating capacity was studied in albino rats which had been given large amounts of phenacetin, NAPA or acetylsalicylic acid for 41—42 weeks. There was a marked and significant decrease of concentrating capacity in the phenacetin and NAPA groups as compared with a control group given no drug. There was a slight but not significant decrease of concentrating capacity in the acetylsalicylic acid group. No definite difference in the urinary sediments could be demonstrated between the groups with or without drug intake.

Acknowledgement

This investigation was supported by grants from Draco, Lunds Farmacevtiska AB.

The technical assistance of Mrs. Elly Lönnerstedt is highly appreciated.

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Salicylates are the most common of all analgesics and are usually assumed in the clinic to be non toxic when given in therapeutic doses. In a clinical material of chronic pyelonephritis with a high frequency of renal papillary necrosis, excessive use of phenacetin was admitted by 60 per cent of the patients, but there were no patients who had taken salicylates daily for years (3) Nor has this study pointed to a nephrotoxic effect from salicylates, the acetylsalicylic acid group not differing significantly from the control group.

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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Morphology of the Gastric Mucosa in Diabetics

By

Bohumil Fixa, Olga Komárková, Vladimír Herout and Jiří Kos

Disturbances of gastric secretion in diabetics have often been reported (e.g. 2). Morphological changes of the gastric mucosa have not yet been evaluated in detail. In this paper we will present the incidence and severity of morphological changes in the gastric mucosa for a considerable number of diabetics.

Methods and material

In 100 diabetic out-patients gastric mucosa was examined in biopsies. The examination was carried out on an empty stomach by means of flexible biopsy tube. There have never been any complications. Specimens of the gastric mucosa were taken from the anterior wall of the gastric body and fixed in 10 % formalin. They were then embedded in paraffin and stained with haematoxylin-eosin, by Hale's method in Muller's modification and by the impregnation method after Foot. For detecting the fat in vacuoles the staining with Sudan III was used.

By the same procedure there was examined control group of 121 non-diabetic patients. This group included patients of the same age as diabetics without any disease of the alimentary tract.

Histological findings were classified into 4 groups:

1. Normal findings (fig. 1)
2. Superficial gastritis (fig. 2)
3. Atrophic gastritis (figs. 3 and 4),
4. Gastritis of undefined type.

In the group of normal findings were included those with a slight infiltrate in the superficial layer. Some authors classify them as the so-called "still normal findings" (5). Histological findings of atrophic gastritis with moderate or marked reduction of the glands were united into one group. The group of gastritis of undefined type comprised specimens in which obvious signs of inflammation were proved, but which could not be characterised more precisely owing to a technical fault in the processing.

The relations between chronic gastritis and age, seriousness, duration and treatment of diabetes were studied in detail.

The seriousness of diabetes was estimated as follows: we considered as mild diabetics those who were compensated on diet alone. Diabetes of moderate to medium seriousness included patients who did well on peroral antidiabetic drugs or insulin at a daily dosage not exceeding 36 units. Diabetes with considerably varying glycosuria and glycemia, often accompanied by ketoacidosis the compensation of which required administration of large doses of insulin (40 or more units daily) was consid-

Submitted for publication July 22, 1963.

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superficial gastritis and 10 patients with atrophic gastritis. Intestinal metaplasia was found in 4 patients with atrophic gastritis. In 5 patients fat vacuoles were demonstrated in the interstitial tissue between the glands (fig 5)

Table III shows the dependence of gastritis on age.

From the findings it is obvious that the difference between the age of the patients with superficial gastritis and that of patients with normal gastric mucosa is statistically significant, as well as that between patients with atrophic gastritis and those with a normal finding

In table IV we can see the dependence of the occurrence of gastritis on the seriousness of diabetes. It follows that the seriousness of diabetes does not influence changes in the gastric mucosa. We do not dare to evaluate minor differences, owing to the small number of examinations.

Table V shows the dependence of the morphologic character of the gastric mucosa on the duration of diabetes. Differences in the duration of diabetes are not statistically significant. Thus the duration of diabetes does not influence the development of morphological changes in the gastric mucosa.

The effect on the gastric mucosa of the method of treatment can be seen in table VI Our results show that the method of treatment does not influence the occurrence of gastritis in diabetics.

Table I. The occurrence of different types of gastritis in diabetics

	No. of cases
Normal gastric mucosa	35 (35%)
Superficial gastritis	24 (24%)
Atrophic gastritis	27 (27%)
Gastritis of undefined type	14 (14%)
	100 (100%)

Table II The occurrence of different types of gastritis in control group

	Average age (yrs)	No. of cases
Normal gastric mucosa	50.1	65 (53.7%)
Superficial gastritis	62.5	25 (20.7%)
Atrophic gastritis	62.4	31 (25.6%)
		121 (100%)

Table III The dependence of gastritis on age

	Average age (yrs)	SD	P
Normal gastric mucosa	54.3	±14.9	–
Superficial gastritis	63.4	± 8.0	<0.01
Atrophic gastritis	62.0	±10.2	<0.05

Discussion

Gastritis was proved through biopsy in 65 of the total number of 100 diabetic patients examined. It is very difficult to estimate whether this means an increased occurrence of gastritis or a normal occurrence in clinically normal subjects of the same age, as we know nothing about the incidence of the gastritis in such subjects. We have shown that the gastritis is not more frequent in diabetics than in non-diabetic patients without any disease of the alimentary tract.

In 15 % of the patients we found signs of acute exacerbation of chronic gastritis. In a previous report we have shown that this finding is not in agreement with the dyspepsia in diabetics (4) In 4 patients



Fig 1 Normal gastric mucosa (hematoxylin and eosin × 100)



Fig 2. Superficial gastritis (hematoxylin and eosin × 100)



Fig 3. Moderate chronic atrophic gastritis (hematoxylin and eosin × 100)

ered as of serious degree The significance of the difference between the incidence of the gastritis in diabetics and that in the control group was studied by the χ test



Fig 4 Marked chronic atrophic gastritis with intestinal metaplasia and fat vacuoles in the basal layer of the mucosa (hematoxylin and eosin × 100)



Fig. 5. Chronic atrophic gastritis with fat vacuoles (hematoxylin and eosin × 200)

Results

Among 100 diabetics examined marked chronic gastritis was proved in 65 cases, i.e. in 65 The occurrence of different types of gastritis is mentioned in table I

The incidence of gastritis in the control group we can see in table II

The total incidence of gastritis and the incidences of the different types of gastritis in diabetics do not significantly differ from those for the patients in the control group ($P < 0.1$)

Signs of acute exacerbation of chronic gastritis were present in 5 patients with

in 3 cases atrophic gastritis and never gastric atrophy the authors thus proved chronic gastritis in 60 %. From our point of view the one salient paper concerned with changes of the gastric mucosa in diabetics, is that of Angervall et al. (1) These authors, however do not show the frequency of occurrence of gastritis in diabetics. They made biopsy examination of a selected group of 13 patients in 8 diabetics with achlorhydria proved by the so-called maximal histamine test they always found atrophic gastritis, while in 5 patients with normochlorhydria they always proved normal gastric mucosa. These findings are in agreement with our experience concerning correlation of secretory changes with the morphology of the gastric mucosa (3)

Summary

Gastric mucosa in 100 diabetics was examined through biopsy In 65 patients, i.e. in 65 %, chronic gastritis was proved. In 24 cases it was superficial gastritis (24 %) in 27 cases (27 %) atrophic gastritis, in 14 diabetics (14 %) gastritis of undefined type. In 35 patients (35 %) gastric mucosa was normal. Gastritis in diabetics was not more frequent than in the control group of 121 non-diabetic patients of the same age without any disturbance of the alimentary tract. In 15 of the patients the signs of acute exacerbation of chronic gastritis and in 5 patients fat vacuoles in gastric mucosa were present. We did not find any relation between chronic gastritis and the severity duration or treatment of diabetes. The gastritis is more frequent in diabetics of higher age. The difference in age between the patients with normal mucosa and those with superficial gastritis as well as those with atrophic gastritis was statistically significant.

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Table IV The dependence of the occurrence of gastritis on the seriousness of diabetes

	Seriousness		
	Mild	Medium	Severe
Normal gastric mucosa	6 (40.0%)	19 (33.3%)	10 (35.7%)
Superficial gastritis	3 (20.0%)	15 (26.3%)	6 (21.4%)
Atrophic gastritis	5 (33.3%)	15 (26.3%)	7 (25.0%)
Gastritis of undefined type	1 (6.7%)	8 (14.1%)	5 (17.9%)

Table V The dependence of gastritis on the duration of diabetes

	Average duration (yrs)	SD
Normal gastric mucosa	4.2	± 3.5
Superficial gastritis	4.7	± 3.7
Atrophic gastritis	5.4	± 3.8

with atrophic gastritis intestinal metaplasia was proved. Fat vacuoles found in 3 patients with atrophic gastritis and in 2 patients with superficial gastritis have not hitherto been described by other authors and their etiology is unknown.

It has been shown in the present study that the frequency of occurrence of gastritis is uncorrelated with the duration, seriousness or the method of treatment of diabetes. We have found a statistically significant difference in age between the patients with normal mucosa and those with superficial gastritis as well as those with atrophic gastritis. This finding points to a more frequent incidence of chronic gastritis with higher age in diabetics.

When considering the etiology of gastritis, one should not forget the frequent occurrence of arteriosclerosis in diabetics although no causal connection between arteriosclerosis and chronic gastritis has so far been confirmed. Decreased resistance of tissues to inflammation in diabetes mellitus is also a possible factor in the etiology of chronic gastritis.

Literature reports on the morphology of the gastric mucosa in diabetics are scanty and are based on the observation of a small number of patients. Joske et al. (6) briefly mention findings in 20 diabetics. In 8 of them normal gastric mucosa was found, in 4 cases there was moderate or serious superficial gastritis, in 5 cases superficial gastritis with atrophy

Table VI The dependence of gastritis on the method of treatment

	Diet	Peroral antidiabetic drugs	Insulin
Normal gastric mucosa	6 (40.0%)	18 (34.6%)	11 (33.3%)
Superficial gastritis	3 (20.0%)	14 (26.9%)	7 (21.2%)
Atrophic gastritis	5 (33.3%)	14 (26.9%)	8 (24.3%)
Gastritis of undefined type	1 (6.7%)	6 (11.6%)	7 (21.2%)
	15 (100 %)	52 (100 %)	33 (100 %)

in 3 cases atrophic gastritis and never gastric atrophy the authors thus proved chronic gastritis in 60 %. From our point of view the one salient paper concerned with changes of the gastric mucosa in diabetics, is that of Angervall et al. (1) These uthors, however do not show the frequency of occurrence of gastritis in diabetics. They made biopsy examination of a selected group of 13 patients in 8 diabetics with achlorhydria proved by the so-called maximal histamine test they always found atrophic gastritis, while in 5 patients with normochlorhydria they always proved normal gastric mucosa. These findings are in agreement with our experience concerning correlation of secretory changes with the morphology of the gastric mucosa (3)

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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The Effects of Aramine® (Metaraminol) on the Splanchnic, the Cardiopulmonary and the Systemic Circulation in Patients with Mitral Valvular Disease

By

Harald Eliasch, Robert O. Malmborg, Bengt Pernow and Staffan Zetterquist

Aramine is a synthetic sympathomimetic amine with vasopressor properties. It exerts a positive inotropic effect on the heart muscle and induces peripheral vasoconstriction (17).

Vasopressor agents such as Aramine are widely used for the treatment of cardiogenic shock in myocardial infarction to raise the systemic blood pressure (15). Whether this is accomplished through an increased cardiac output in the face of unaltered peripheral vascular resistance and/or through augmented peripheral vasoconstriction is a matter of great interest. Major technical difficulties have so far made the patient in cardiogenic shock inaccessible to thorough hemodynamic investigation of this problem.

In this paper the effects of Aramine were studied in patients with mitral valvular disease. Besides investigating cardiopulmonary hemodynamics, the splanchnic blood flow was also estimated, which, because of its significant contribution to the cardiac output must play an important role in the regulation of the circulatory homeostasis in the body.

Material

The studies were made on eight patients with mitral valvular disease, three females and five males. Their ages ranged between 43 and 55 years. When grouped according to the criteria of the New York Heart Association (7) most patients fell into group III. Six patients had atrial fibrillation. The heart volume (12) ranged between 580 and 910 ml/sqm body surface area. Clinical data are summarized in table I.

Methods

The patients were studied in the morning in the postabsorptive state. Right heart catheterization was performed in the usual manner (11) using a double lumen catheter. A second catheter was introduced percutaneously into the right femoral vein (10, 21) and its tip manipulated into the main right hepatic vein (5). A teflon catheter was introduced into the brachial artery and another into a cubital vein.

Submitted for publication July 23, 1963.

Table I Clinical data, heart volume by roentgen hematocrit and blood volume derived by the BSP single injection technic and the carbon-monoxide method respectively (SR = sinus rhythm Af = atrial fibrillation)

Case no.	Sex	Age	BSA (m^2)	Group	Rhythm	Heart vol. (ml/m)	Hct (%)	Blood vol. (l)	
								BSP	carb. monox.
6	♀	55	1 72	III	Af	670	47	3.2	3.4
13	♂	51	1.89	III	Af	670	40	6.0	6.6
14	♂	55	1.80	III	Af	720	38	3.7	3.6
16	♂	51	1.83	III	Af	910	44	4.1	5.1
17	♂	43	1 96	III	Af	720	44	6.1	5.7
18	♀	54	1 70	III	SR	480	40	4.5	4.2
19	♀	47	1 76	II	SR	380	40	3.7	3.5
20	♂	51	1.86	III	Af	720	45	5.0	3.7

Pressures in the right heart and the brachial artery were measured by the strain gauge manometer and inscribed on the oscillograph. The cardiac output was determined by the Fick method, mixed venous blood being sampled from the main stem of the pulmonary artery

The splanchnic blood flow was estimated by the method of Bradley et al. (4) A detailed description of this technique including minor modifications has recently been reported elsewhere (5 6)

100 mg of bromsulphalein (BSP) was injected into the cubital vein. Peripheral blood samples were obtained after 3 5 7 and 9 minutes. The BSP space was calculated by extrapolation to zero time (5, 6)

Thereafter a priming dose of 100 mg BSP was administered and then BSP was infused by a motor-driven syringe, delivering 3 to 4 mg of the dye per kilogram body weight. This rate of infusion was used except for studies No. 18, 19 and 20, where the amount of BSP was reduced by 40 % as the Aramine infusion was started. Blood samples for the BSP determination were obtained from the hepatic vein and the brachial artery at 4–10 minutes intervals throughout the study. BSP was analyzed according to Gaebler's method (9)

Blood glucose lactate and pyruvat were determined on samples obtained from the hepatic vein the pulmonary and the brachial arteries. The methods of Mark (14) Barker and Summerson (1) and Friedmann and Haugen (8) respectively were used

The blood volume was measured by the carbon monoxide method according to Sjöstrand (18)

Calculations

$$\text{ESBF ml/min} \quad \frac{\text{Removal}}{p - h} \times \frac{100 \times 100}{100 - \text{Hct}}$$

$$\text{Removal} = I \pm \frac{\Delta p \times 0.8 \times \text{BSP space}}{100}$$

SOC ESBF × A – HvO diff

ESBF Estimated splanchnic blood flow

I = Infused amount of BSP in mg/min.

p = Arterial concentration of BSP in mg/100 ml plasma.

h = Hepatic venous concentration of BSP in mg/100 ml plasma.

Δp = Change in arterial concentration of BSP in mg/100 ml plasma per minute.

Hct = Hematocrit.

SOC = Splanchnic oxygen consumption in ml

A–HvO diff = Arterio-hepatic venous oxygen difference in ml/100 ml

Procedure

Samples for the determination of the splanchnic blood flow the cardiac output and blood glucose, lactate and pyruvate were obtained when the patient had rested for 30 minutes. Thereafter a drip containing 50 mg of Aramine in 500 ml of isotonic saline solution was started via the heart catheter in the right ventricle, at a rate such that 0.1–0.3 mg Aramine was delivered per minute. Pressures in the pulmonary circuit and the peripheral artery were measured repeatedly at short intervals.

Table II Arterial and hepatic venous bromsulphalein plasma-concentration in mg/100 ml, estimated splanchnic blood flow, splanchnic oxygen data, the splanchnic share of the cardiac output (CO) and the splanchnic vascular resistance in eight patients with mitral valvular disease. Mean values obtained at rest (R) and on infusion of Aramine (A)

Case no.		BSP			ESBF mean (ml/m²)	Splanchnic A-V O₂ diff. (ml/l)	Splanchnic O₂ cons. (ml/m²)	ESBF / CO (%)	Splanchnic vasc. resist. (dyn sec cm⁻⁵)
		Range art conc.	Range (Δp mg/100 ml/min)	Range hep. conc.					
6	R	1.84–1.99	−0.010–+0.003	1.21–1.50	706	69	49	34	910
	A	1.58–1.96	+0.015–+0.058	0.85–1.24	727	70	51	22	950
13	R	0.78–0.84	−0.012–+0.006	0.24–0.32	603	73	44	24	330
	A	0.81–1.09	+0.007–+0.032	0.28–0.45	460	103	47	17	1,120
14	R	0.97–1.03	−0.015–+0.002	0.52–0.69	556	71	39	20	760
	A	1.02–1.22	+0.001–+0.021	0.52–0.70	478	98	47	19	1,660
16	R	1.10–1.13	±0 –+0.006	0.32–0.36	426	47	20	22	910
	A	1.11–1.37	−0.002–+0.027	0.35–0.67	388	59	23	16	1,330
17	R	1.12–1.14	−0.002–+0.002	0.42–0.55	531	51	27	28	730
	A	1.36–1.59	+0.011–+0.034	0.50–0.55	332	111	37	14	1,600
18	R	0.97–0.99	−0.004–+0.001	0.56–0.63	482	65	31	17	800
	A	0.92–0.94	−0.004–±0	0.48–0.59	424	71	30	11	1,220
19	R	2.11–2.34	−0.020–+0.002	1.48–1.55	574	45	26	16	750
	A	1.91–2.04	−0.028–+0.030	1.38–1.52	381	72	27	11	1,980
20	R	1.59–1.74	−0.032–+0.002	0.56–1.04	565	70	40	26	600
	A	1.26–1.30	−0.004–±0	0.50–0.70	457	95	43	20	990

After 5–10 minutes a significant rise in the brachial arterial pressure was usually noted, associated with a decrease in the heart rate. At this time measurement of the cardiac output was started again and samples for determining the other mentioned chemical parameters obtained.

The Aramine drip had to be discontinued in the majority of studies before the end of blood sampling. The reason for this was that ventricular extrasystoles and/or headache developed. These side effects disappeared shortly after.

Results

1 Splanchnic circulation and metabolism

The *arterial concentration of BSP* as measured at least 20 minutes after the onset of the continuous drip of the dye, was fairly constant when the patient was at rest. The change in concentration (Δ p) showed variations usually between ± 0.006 mg/100 ml/minute but occasionally higher. During the infusion of Aramine, a steep rise in Δ p was noted and splanchnic extraction of dye decreased. In studies No 18 and 20 where the infused amount of dye was intentionally reduced, the change in Δ p was smaller (table II).

The *estimated splanchnic blood flow* (ESBF) varied at rest between 482 and 706 ml/min./sqm body surface area. When Aramine was given, the ESBF save for one study (Case [illegible]) decreased to varying degrees (table II fig. 4).

Table I Clinical data, heart volume by roentgen, hematocrit and blood volume derived by the BSP single injection technique and the carbon-monoxide method respectively (SR = sinus rhythm Af = atrial fibrillation)

Case no.	Sex	Age	BSA (m)	Group	Rhythm	Heart vol (ml/m)	Hct (%)	Blood vol. (l) BSP	carb. monox.
6	♀	55	1 72	III	Af	670	47	3.2	3.4
13	♂	51	1.89	III	Af	670	40	6.0	6.6
14	♂	55	1.80	III	Af	720	38	3.7	3.8
16	♂	51	1.83	III	Af	910	44	4 1	5.1
17	○	43	1.96	III	Af	720	44	6.1	5.7
18	♀	54	1 70	III	SR	480	40	4.5	4.2
19	♀	47	1 76	II	SR	380	40	3.7	3.5
20	♂	51	1.86	III	Af	720	45	5.0	5.7

Pressures in the right heart and the brachial artery were measured by the strain gauge manometer and inscribed on the oscillograph. The cardiac output was determined by the Fick method, mixed venous blood being sampled from the main stem of the pulmonary artery

The splanchnic blood flow was estimated by the method of Bradley et al. (4) A detailed description of this technique including minor modifications has recently been reported elsewhere (5 6)

100 mg of bromsulphalein (BSP) was injected into the cubital vein. Peripheral blood samples were obtained after 3 5 7 and 9 minutes. The BSP space was calculated by extrapolation to zero time (5 6)

Thereafter a priming dose of 100 mg BSP was administered and then BSP was infused by a motor-driven syringe, delivering 3 to 4 mg of the dye per kilogram body weight. This rate of infusion was used except for studies No. 18 19 and 20 where the amount of BSP was reduced by 40 % as the Aramine infusion was started. Blood samples for the BSP determination were obtained from the hepatic vein and the brachial artery at 4–10 minutes intervals throughout the study BSP was analyzed according to Gaebler's method (9)

Blood glucose, lactate and pyruvate were determined on samples obtained from the hepatic vein, the pulmonary and the brachial arteries. The methods of Mark (14) Barker and Summerson (1) and Friedmann and Haugen (8) respectively were used.

The blood volume was measured by the carbon monoxide method according to Sjöstrand (18)

Calculations

$$\text{ESBF ml/min} = \frac{\text{Removal}}{p - h} \times \frac{100 \times 100}{100 - \text{Hct}}$$

$$\text{Removal} = I \pm \frac{\Delta p \times 0.8 \times \text{BSP space}}{100}$$

SOC = ESBF × A – HvO_2 diff.

ESBF = Estimated splanchnic blood flow
I = Infused amount of BSP in mg/min.
p = Arterial concentration of BSP in mg/100 ml plasma.
h = Hepatic venous concentration of BSP in mg/100 ml plasma.
Δp = Change in arterial concentration of BSP in mg/100 ml plasma per minute.
Hct = Hematocrit.
SOC = Splanchnic oxygen consumption in ml
A – HvO_2 diff = Arterio-hepatic venous oxygen difference in ml/100 ml.

Procedure

Samples for the determination of the splanchnic blood flow the cardiac output and blood glucose lactate and pyruvate were obtained when the patient had rested for 30 minutes. Thereafter a drip containing 50 mg of Aramine in 500 ml of isotonic saline solution was started via the heart catheter in the right ventricle, at a rate such that 0.1–0 3 mg Aramine was delivered per minute. Pressures in the pulmonary circuit and the peripheral artery were measured repeatedly at short intervals.

Table III. Glucose, lactate and pyruvate concentration in plasma of the hepatic vein, the brachial artery (BA) and the pulmonary artery (PA). The splanchnic uptake (negative values) and output (positive values) in mg/min/m BSA. Observations were made at rest (R) and on infusion of Aramine (A) in six patients with mitral valvular disease

Case no.		Glucose (mg/100 ml)			Lactate (mg/100 ml)			Pyruvate (mg/100 ml)			Splanchnic uptake/output		
		Hep.	BA	PA	Hep.	BA	PA	Hep.	BA	PA	Glucose	Lactate	Pyruvate
13	R	127	118	115	5.7	10.3	12.3	0.72	1.11	1 10	− 54.2	27 7	2.35
	A	138	129	125	11 4	14.0	12.5	0.80	1.17	1 16	−133 4	12.0	1 70
14	R	101	100	88	6.7	14.8	11 7	1.08	1 17	1.23	− 5.6	43.0	0.50
	A	110	91	94	8.2	10.7	12.3	1.54	1.29	1.36	− 90.8	56.3	0.76
17	R	126	98	91	3.8	3.9	3.0	0.57	0.64	0.63	−148.8	0.5	0.37
	A	106	80	86	2.1	3.9	4.0	0 65	0.62	0.68	− 86.3	6.0	−0.10
18	R	115	115	103	9.0	8.3	9.8	0.89	0.96	0.87	0	1 4	0.33
	A	124	108	109	7 4	9.2	9.0	1 12	0.97	0.95	63 6	15.7	−0.04
19	R	105	94	93	7.5	5.5	9.5	0.69	0.75	0.76	− 83.1	−11.5	0.34
	A	120	103	93	2.5	5.0	7.0	0.69	0.68	0.66	− 64.7	9.5	−0.05
20	R	108	83	84	4.2	7.1	7 1	–	0.80	0.81	141.3	16.4	–
	A	112	89	88	4.0	8.0	10 0	0.56	0 82	0.82	−103 0	18.2	1.09

The *splanchnic arteriovenous difference of glucose, pyruvate and lactate* was measured in five studies. These differences varied at random in the resting state and no consistent change was noted when Aramine was given (table III).

II. Pulmonary and systemic circulation

The *heart rate* initially decreased as the systemic arterial pressure rose. In those studies where the Aramine infusion had to be discontinued, the heart rate increased again before the flow measurements were made. The *arterial pressure* was still increased in these patients (table IV).

In seven of the eight studies, the *total oxygen consumption* increased when Aramine was given. The rise varied between 9 and 48 per cent (table IV).

The *systemic arteriovenous oxygen difference* varied between 28 and 64 ml/l at rest. On Aramine, no significant change was obtained.

The *cardiac output* varied between 1.9 and 3 6 l/min./sqm body surface area. On Aramine, the output increased in four studies, remaining essentially unchanged in the others. The increase in the cardiac output was largest in those two studies where the arteriovenous oxygen difference over the pulmonary circuit decreased (table IV).

The *splanchnic share of the cardiac output* ranged between 16 and 34 per cent when the patient was at rest. On Aramine, this ratio invariably decreased to values between 11 and 22 per cent. This decrease was not quantitatively correlated either to pressures in the pulmonary artery or the brachial artery or to the end-diastolic right ventricular pressure (table IV).

The mean *brachial arterial* pressure rose to varying levels when Aramine was given.

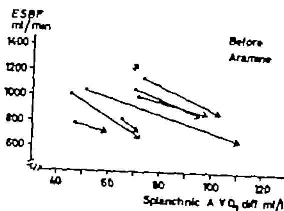


Fig 1 This graph illustrates the relationship between the estimated splanchnic blood flow and the splanchnic arterio-venous oxygen difference in 8 patients with mitral valvular disease Observations were made before and on infusion of Aramine.

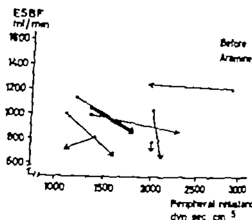


Fig 3 This graph illustrates the relationship between the estimated splanchnic blood flow and the peripheral vascular resistance in 8 patients with mitral valvular disease Observations were made before and on infusion of Aramine.

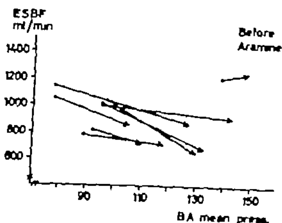


Fig 2. This graph illustrates the relationship between the estimated splanchnic blood flow and the mean brachial arterial pressure in mm Hg in 8 patients with mitral valvular disease. Observations were made before and on infusion of Aramine.

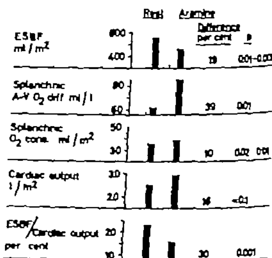


Fig 4 This graph shows means and differences of the estimated splanchnic blood flow the splanchnic arterial-venous oxygen difference, the splanchnic oxygen consumption, the cardiac output and the splanchnic share of the cardiac output, derived from observations made at rest and on infusion of Aramine Studies were made in 8 patients with mitral valvular disease

The *oxygen difference* between the artery and the hepatic vein (A HvO diff) varied between 45 and 73 ml/l at rest On Aramine a rise was invariably observed, the values ranging between 59 and 103 ml/l The change in this arteriovenous oxygen difference correlated inversely to the alterations in the ESBF (table II fig 1)

The calculated *splanchnic oxygen consumption* varied at rest between 20 and 49 ml/sqm body surface area. Generally a small rise was registered during the infusion of Aramine (table II fig 4)

Table III Glucose, lactate and pyruvate concentration in plasma of the hepatic vein, the brachial artery (BA) and the pulmonary artery (PA). The splanchnic uptake (negative values) and output (positive values) in mg/min/m² BSA. Observations were made at rest (R) and on infusion of Aramine (A) in six patients with mitral valvular disease

Case no.	Glucose (mg/100 ml)			Lactate (mg/100 ml)			Pyruvat (mg/100 ml)			Splanchnic uptake/output		
	Hep.	BA	PA	Hep. v	BA	PA	Hep.	BA	PA	Glucose	Lactate	Pyruvat
13 R	127	118	115	5.7	10.5	12.3	0.72	1 11	1 10	− 54.2	27 7	2.35
A	158	129	125	11.4	14.0	12.5	0.80	1.17	1 16	−133.4	12.0	1 70
14 R	101	100	88	6.7	14.8	11 7	1.08	1 17	1.23	− 5.6	45.0	0.50
A	110	91	94	8.2	10.7	12.5	1.54	1.29	1.36	− 90.8	36.3	0.78
17 R	126	98	91	3.8	3.9	3.0	0.57	0 64	0.65	−148.6	0 5	0.57
A	106	80	86	2.1	3.9	4.0	0.65	0.62	0.68	− 86.3	6.0	−0.10
18 R	115	115	103	8.0	9.3	9.8	0.89	0.96	0.87	0	1 4	0.53
A	124	109	109	7 4	9.2	9.0	1 12	0.97	0.95	63.6	15.7	−0.04
19 R	105	94	95	7.3	5.5	9.5	0.69	0.75	0.78	− 63.1	−11.5	0.34
A	120	103	94	2.5	5.0	7.0	0.60	0.68	0 66	− 64.7	9.5	−0 03
20 R	108	83	84	4.2	7 1	7.1	—	0.80	0.81	141.3	16.4	—
A	112	89	86	4 0	8.0	10 0	[illegible]	0.82	0.82	−105 0	18.2	1.09

The *splanchnic arteriovenous difference of glucose pyruvate and lactate* was measured in five studies. These differences varied at random in the resting state and no consistent change was noted when Aramine was given (table III)

II Pulmonary and systemic circulation

The *heart rate* initially decreased as the systemic arterial pressure rose. In those studies where the Aramine infusion had to be discontinued, the heart rate increased again before the flow measurements were made. The arterial pressure was still increased in these patients (table IV)

In seven of the eight studies, the *total oxygen consumption* increased when Aramine was given. The rise varied between 9 and 48 per cent (table IV)

The *systemic arteriovenous oxygen difference* varied between 28 and 64 ml/l at rest. On Aramine, no significant change was obtained.

The *cardiac output* varied between 1.9 and 3 6 l/min./sqm body surface area. On Aramine, the output increased in four studies, remaining essentially unchanged in the others. The increase in the cardiac output was largest in those two studies where the arteriovenous oxygen difference over the pulmonary circuit decreased (table IV)

The *splanchnic share of the cardiac output* ranged between 16 and 34 per cent when the patient was at rest. On Aramine, this ratio invariably decreased to values between 11 and 22 per cent. This decrease was not quantitatively correlated either to pressures in the pulmonary artery or the brachial artery or to the end-diastolic right ventricular pressure (table IV)

The mean *brachial arterial* pressure rose to varying levels when Aramine was given.

Table IV Heart rate cardiac output (CO) pressures (mm Hg) in the pulmonary capillaries (PCV) the pulmonary artery (PA) the right ventricle (RV) and the brachial artery (BA) and the pulmonary and peripheral vascular resistance in eight patients with mitral valvular disease. Observations were made at rest (R) and on infusion of Aramine (A) (S = systolic, D = diastolic M = mean)

Case no.		Heart rate	O cons. (ml)	AVO diff (ml/l)	CO (l/m)	PCV mean	PA			RV		BA			Resistance	
							S	D	M	S	D	S	D	M	Pulm.	Peripher.
6	R	70	218	61	2 1	28	48	30	36	47	8	197	100	138	180	2,900
	A	87	274	48	3.3	35	79	38	53	—	8	204	112	148	250	2,900
13	R	63	254	53	2.5	27	47	29	36	47	8	106	63	78	150	1,200
	A	87	333	67	2.6	44	100	50	68	98	14	200	92	127	385	1,800
14	R	54	253	50	2.8	29	55	27	38	59	8	136	80	95	140	1,350
	A	45	276	60	2.6	31	91	32	46	89	8	261	106	143	260	2,300
16	R	83	207	57	2.0	18	40	15	25	38	0	119	70	89	160	2,000
	A	96	306	69	2.4	—	68	32	47	68	9	151	93	118	—	2,000
17	R	75	220	59	1.9	16	27	19	23	27	4	123	78	97	150	2,000
	A	54	262	38	2.3	—	62	35	45	67	10	191	102	130	—	2,100
18	R	87	223	47	2.8	33	58	35	42	50	11	128	74	92	130	1 400
	A	84	273	40	4 0	—	74	46	55	70	14	163	87	110	—	1,100
19	R	84	180	28	3.6	15	30	15	20	38	5	147	72	95	60	1 100
	A	66	236	40	3.4	19	45	20	29	50	10	193	90	133	130	1 600
20	R	51	265	64	2.2	30	52	27	41	48	9	108	65	78	210	1,350
	A	51	254	60	2.3	36	74	42	51	74	11	157	79	105	290	1,800

This rise varied between 7 and 63 per cent The pressure increment was associated with a decrease in the ESBF There was no correlation between the changes in pressure and the peripheral vascular resistance (table IV fig 2)

The *peripheral vascular resistance* rose in four studies, being unchanged or decreased in the two others when Aramine was given. There was no constant relationship between the change in the peripheral vascular resistance and the alterations in the ESBF (table IV fig 3)

The *pulmonary capillary venous* pressure (PCV) varied at rest between 15 and 33 mm Hg On Aramine, as measured in five studies, a moderate rise was registered (table IV)

The mean *pulmonary arterial* pressure varied between 20 and 41 mm Hg On Aramine, the pressure increased the rise varying between 21 and 96 per cent. The pulse pressure rose in all studies (table IV)

The *pulmonary vascular resistance* was within normal range at rest. On Aramine (as calculated in five studies) the resistance increased in four studies to values exceeding 200 dyn./sec./cm^{-5} (table IV)

The *right ventricular end-diastolic* pressure varied between 0 and 11 mm Hg On Aramine, this pressure level was raised in

6 studies. The pressure change was not correlated to the alterations in the cardiac output (table IV)

Means of differences of the measured parameters are shown in figs. 4 and 5 The course of events in one study No. 17 is graphically illustrated in fig 6

Discussion

The measurement of pressures and flow at the time when the vasopressor effects of Aramine became obvious could not be made in steady-state conditions. This was mainly due to a sudden and often unpredicted onset of action of the drug This disadvantage was not alleviated by administering relatively small amounts. In some studies side effects, such as headache and/or a sensation of chill, also appeared. In these instances, the infusion of Aramine was promptly discontinued. However it was noticed that once the systemic arterial blood pressure increased, the rise was maintained for sufficient period of time to allow of repeated sampling of blood and recording of pressures. As the present results were compiled from means of several observations, showing only moderate variation, it was believed that sufficient confidence could be placed in the data.

The validity of measuring the splanchnic blood flow by the bromsulphalein method rests on certain requirements One is that the peripheral plasma concentration of the dye must be held at a nearly constant level (4) This condition is ordinarily satisfied when as in the present study the patient is investigated in the resting state and the liver function is not seriously impaired (4) However in the face of a change in the splanchnic blood flow the hepatic remov

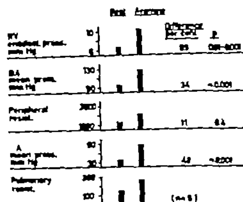


Fig 5. This graph shows means and differences of the right ventricular end-diastolic pressure the brachial arterial mean pressure, the peripheral vascular resistance, the pulmonary arterial mean pressure and the pulmonary vascular resistance, derived from observations made at rest and on infusion of Aramine. Studies were made in 8 patients with mitral valvular disease

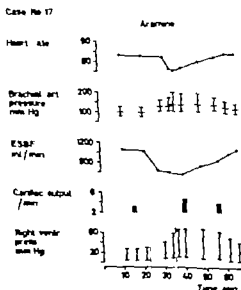


Fig 6. This graph illustrates the course of hemodynamic events in patient with mitral valvular disease studied at rest, before and after infusion of Aramine.

Table IV Heart rate cardiac output (CO) pressures (mm Hg) in the pulmonary capillaries (PCV) the pulmonary artery (PA) the right ventricle (RV) and the brachial artery (BA) and the pulmonary and peripheral vascular resistance in eight patients with mitral valvular disease Observations were made at rest (R) and on infusion of Aramine (A) (S = systolic D = diastolic M = mean)

Case no.		Heart rate	O cons. (ml)	A VO diff. (ml/l)	CO (l/m.)	PCV mean	PA			RV		BA			Resistance	
							S	D	M	S	D	S	D	M	Pulm.	Peripher.
6	R	70	218	61	2 1	28	48	30	36	47	8	197	100	138	180	2,900
	A	87	274	48	3.3	35	79	38	53	—	8	204	112	148	250	2,900
13	R	63	254	53	2.5	27	47	29	36	47	8	106	63	78	150	1,200
	A	87	333	67	2.6	44	100	50	68	98	14	200	92	127	385	1,800
14	R	54	253	50	2.8	29	55	27	38	59	8	136	80	95	140	1,350
	A	45	276	60	2.6	31	91	32	46	89	8	261	106	143	260	2,300
16	R	83	207	57	2.0	18	40	15	25	38	0	119	70	89	160	2,000
	A	96	306	69	2.4	—	68	32	47	68	9	151	93	118	—	2,000
17	R	75	220	59	1.9	16	27	19	23	27	4	123	78	97	150	2,000
	A	54	262	58	2.3	—	62	35	45	67	10	191	102	130	—	2,100
18	R	87	223	47	2.8	33	58	35	42	50	11	128	74	92	150	1,400
	A	84	273	40	4 0	—	74	46	55	70	14	165	87	110	—	1,100
19	R	84	180	28	3.6	15	30	15	20	38	5	147	72	95	60	1,100
	A	66	236	40	3.4	19	45	20	29	50	10	193	90	133	130	1,600
20	R	51	265	64	2.2	30	52	27	41	48	9	108	65	78	210	1,350
	A	51	254	60	2.3	36	74	42	51	74	11	157	79	105	290	1,800

This rise varied between 7 and 63 per cent The pressure increment was associated with a decrease in the ESBF There was no correlation between the changes in pressure and the peripheral vascular resistance (table IV fig 2)

The *peripheral vascular resistance* rose in four studies, being unchanged or decreased in the two others when Aramine was given There was no constant relationship between the change in the peripheral vascular resistance and the alterations in the ESBF (table IV fig 3)

The *pulmonary capillary venous* pressure (PCV) varied at rest between 15 and 33 mm Hg On Aramine, as measured in five studies a moderate rise was registered (table IV)

The mean *pulmonary arterial* pressure varied between 20 and 41 mm Hg On Aramine, the pressure increased the rise varying between 21 and 96 per cent. The pulse pressure rose in all studies (table IV)

The *pulmonary vascular resistance* was within normal range at rest. On Aramine (as calculated in five studies) the resistance increased in four studies to values exceeding 200 dyn./sec./cm^{-5} (table IV)

The *right ventricular end-diastolic* pressure varied between 0 and 11 mm Hg On Aramine, this pressure level was raised in

ed cardiac performance, which agrees with previous observations made in dogs (17)

In the present study it was thus shown that the administration of Aramine resulted in a systemic arterial pressure rise that was associated with splanchnic vasoconstriction, and, in some studies, that this rise was not a consequence of further augmentation in the peripheral vascular resistance. Also, this mode of action of Aramine must be of value in cardiogenic shock, but whether the aforementioned mechanisms will operate in situations where a different set of circulatory circumstances prevail must still be left to conjecture.

On the infusion of Aramine the external work of both ventricles increased. This was paralleled by a rise in the end diastolic right ventricular pressure and the pulmonary capillary venous pressure, which could be explained by increased myocardial contractility and improved ventricular function as earlier suggested (17)

Summary

1 Right heart and right hepatic venous catheterization was performed in eight patients with mitral valvular disease of moderate to marked severity Studies were made at rest and upon the administration of Aramine (metaraminol).

2 The splanchnic blood flow was measured by the bromsulphalein method. It was diminished in the resting state, and Aramine led to a further reduction in flow concomitant with the increase in systemic arterial blood pressure. The possible consequences of splanchnic vasoconstriction are discussed.

3. The rise in systemic arterial pressure, measured after the administration of Aramine, was in four studies paralleled by an increase in the cardiac output. The peripheral vascular resistance was unaltered in these, while it rose in the remaining four cases.

4 The right ventricular end-diastolic and the pulmonary capillary venous pressures tended to rise when Aramine was given. It is believed that these features could be brought about by increased myocardial contractility Thereby an explanation is afforded for the finding of a rise in the systemic arterial pressure despite unaltered peripheral vascular resistance as observed on the infusion of Aramine in some of the studies.

5 The hepatic uptake and output of glucose, lactate and pyruvate was measured. On the infusion of Aramine no evidence of hepatic glycogenolysis was found.

Acknowledgement

This investigation was aided by grants from the Swedish National Foundation against Cardiovascular and Pulmonary Disease. Aramine® is manufactured by Merck Sharp & Dohme Co., Rahway N J., USA.

The technical assistance of Miss B. Ryd, Mrs. A. Liepins, Mrs. K. Tornberg, Mrs. A. Rotschild, Miss B. Hultenberg and Miss K. Sjoberg is gratefully acknowledged.

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al of bromsulphalein becomes altered leading to variations in the peripheral level of the dye In this study the infusion of Aramine was associated with a sharp rise in the dye level It has recently been shown that in such circumstances the splanchnic blood flow will be somewhat overestimated (5) In consequence, the observed fall in the splanchnic blood flow occurring as the arterial pressure rose was rather underestimated To overcome this potential error the rate of infusion of bromsulphalein was reduced in three studies as the infusion of Aramine was started. This resulted in a nearly constant level of dye throughout the whole investigation The reduction of splanchnic blood flow as measured in those cases did not differ substantially from that observed in the others. In addition, the close relationship between the degree of fall in splanchnic blood flow and rise in the splanchic arteriovenous oxygen difference as obtained on Aramine lends further support to the claim that the splanchnic blood was measured with reasonable accuracy

The present material of mitral valvular disease consisted of patients in their fifties, the majority being considerably disabled as judged from the results of the clinical investigation Moderate up to severe pulmonary hypertension associated with a decrease in the cardiac output was accordingly registered The estimated splanchnic blood flow ranging between 706 and 426 ml per square meter body surface area, was subnormal Generally this reduction paralleled the decrease in the cardiac output although the splanchnic share varied at random. The degree of pulmonary hypertension or the level of the right ventricular end-diastolic pressure did not correlate to this ratio. These findings are substantially in agreement with those of others (15 16 19) and may also serve to emphasize that the splanchnic blood flow in heart disease is primarily adjusted to maintain circulatory homeostasis (low cardiac output) and thus is not necessarily reduced as a consequence of right heart failure.

Following the administration of Aramine the changes in flow became more consistent. As the systemic arterial pressure rose, the estimated splanchnic blood flow decreased. These results are in agreement with those earlier reported as to effects of norepinephrine (2) The cardiac output increased or was substantially unchanged, which means that the splanchnic share of the cardiac output decreased. This circulatory adjustment is in several respects similar to that observed during physical exercise (3 19)

Aramine did not significantly influence the peripheral glucose, lactate and pyruvate concentrations. Also in this respect Aramine mimics noradrenaline which has little effect on hepatic glycogenolysis (2)

Since the infusion of Aramine resulted in a rise in arterial pressure and decrease in splanchnic blood flow vasoconstriction was thus elicited in the splanchnic vascular area Thereby blood volume is presumably augmented in central parts of the circulatory system in order to enhance venous return In addition, the redistribution of blood flow will also serve the same purpose. If Aramine will cause a similar circulatory adjustment in circulatory shock remains to be demonstrated

There was no correlation between the rise in arterial pressure and the change in peripheral vascular resistance. In some studies the pressure rise paralleled the increase in the cardiac output without markedly affecting the vascular resistance. This suggests that the pressure augmentation could be the consequence of improv-

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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Studies on Blood Coagulation Factors in a Case of Liver Cirrhosis

Remission of the Hemorrhagic Tendency on Treatment with Heparin

By

Stig-Arne Johansson

Patients with liver cirrhosis have often been found to have an increased fibrinolytic activity in their blood (8 9 12) The increased bleeding tendency in hepatocellular disease has been ascribed by several authors at least partly to this enhanced fibrinolytic activity (9 13 20) The complexity of the picture, including coagulatory and vascular as well as hemodynamic factors, makes it sometimes difficult to ascribe the increased bleeding tendency solely to the increased fibrinolytic activity Thus, multiple deficiencies of coagulation factors are also often a striking finding in this disease (3 20) The development of these deficiencies may be explained by the impaired liver function

In 1960 Bergström et al. (3) in their study of case with liver cirrhosis afforded indirect evidence that the fibrinolysis was secondary to a state of hypercoagulability

In the present paper a case of liver cirrhosis is reported which presented a hemorrhagic tendency and laboratory findings of multiple deficiencies of coagulation factors including platelets. Hypercoagulability was considered to be of importance in the pathogenesis of the condition and the patient was successfully treated with heparin.

Case report

An 85-year-old woman was hospitalized in Oct. 1958 for the first time because of a chronic cholecystitis. In Feb. 1960 a gangrenous gall bladder with stones was excised.

This study started in Oct. 1960 when she was hospitalized because of high fever and a cystitis. X-ray examination of her stomach, esophagus and large intestine did not show any pathological changes. No signs of portal hypertension were found. Biopsy of the liver showed cirrhosis probably due t cholangitis. Paper electrophoresis Oct. 25th showed per 100 ml serum albumin 2 71 g, α_1 0.33 g, α_2 0 43 g, β 0.73 g, γ 3.30 g Thymol turbidity

Reported at Staffmeeting at Södersjukhuset, February 10th, 1961

Submitted for publication July 21 1961

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Table I Different coagulation factors in the patient plasma

	21.11 60	23.11.60	29.11 60	2.12.60	28.11 61	Normal value	Methods
Coagulation time	8'15"	5'35	6 47	5 48'	7'10" 9'45"	4 –8'	Modified Hedenius (10)
In silicone tubes	20'	14 –18'	12 –17	10'–14	12'–26'	20'–30'	Modified Lee & White (15)
Bleeding time	2'30"	3'16	—	2'26"	—	1 –5	Duke (7)
Platelets/μl	85,000	56,000	48,000	45,000	230,000	200,000–400 000	Kristensson (12)
Prothrombin consumption Residual	16	8.2	28	—	—	0–30	Biggs & MacFarlane (5)
Prothrombin + proconvertin (factors II + VII) (%)	47	44	60	44	83	85–110	Owren & Aas (21)
Factor V (%)	78	50	54	44	69	80–120	Wolf (27)
AHF (factor VIII) (%)	143	210	316	193	248	63–135	Nilsson et al. (19)
Factor IX ( )	51	46	34	82	86	60–140	Nilsson et al (19)
Circ. anticoagulants	0	0	0	0	0	0	Prolong. of recalcif. time of normal plasma (19)
Fibrinogen g/100 ml in	0.18	0.19	0.23	0.34	0.44	0.26 ± 0.06	Bergström et al. (3)
Fibrinolysis (μg/hr) at pH 6.3	160	0	0	0	0	31 ± 23	Bergström et al. (3)
pH 7 1	3	0	0	0	0	18 ± 34	

value fell to 5 1 g/100 ml during the following days and low levels for fibrinogen (0.15 g/100 ml and prothrombin + proconvertin activity (2) were noted. On Dec. 20th she was given 300 ml of washed red blood cells and 100 mg of heparin. On the following day she received 600 ml of washed red blood cells and 200 mg of heparin. No adverse reactions were observed and the patient felt quite well. When the patient was treated with washed red cells and pretreated with heparin before the transfusions rapid increase in the hemoglobin values was observed. Heparin (100 mg) was then given daily until Dec. 29th. After heparin treatment

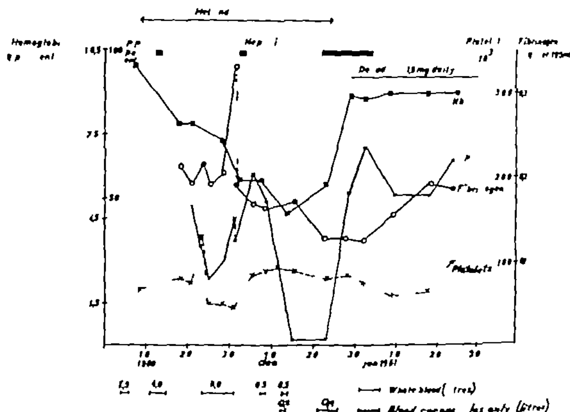


Fig 1 Variations in hemoglobin values, platelet numbers, prothrombin + proconvertin activity fibrinogen concentration and melaena studied during periods of blood transfusion therapy as well as during heparin therapy

test 11—12.5 Maclagan units. On Nov 5th she vomited a pint of blood and melaena was observed

On Nov 12th she once more got shivers and fever vomited about a pint of blood and melaena appeared again during the night. During the following days her blood pressure varied between 80/50 and 130/70 Blood transfusions amounting to 4 l were given on the 11th, 12th, 14th. On Nov 15th she vomited altogether about three pints of blood. In the evening 100 mg heparin (Vitrum) was given intravenously Within two hours after th first injection she felt much better no more vomiting occurred, her skin became warm and dry and the blood pressure rose. The heparin administration was repeated every 6 hours for 24 hours.

On Nov 16th, during heparin therapy she received one pint of blood to increase her hemoglobin value. This time she did not show any untoward reaction (cf below and above)

Because of her low hemoglobin values, 7 l of blood (freshly drawn with silicone technique) was given from Nov 24th to Dec 1st. Despite this therapy her hemoglobin decreased to 6.1 g/100 ml. During this last period of blood therapy her blood pressure decreased to 120—110/70—55 and she got nausea and abdominal pains daily On the evening of the 2nd of Dec. she vomited about a pint of blood. All blood therapy was then stopped and heparin therapy (500 mg during 24 hours) was again instituted with good subjective effect In spite of low hemoglobin values (about 6 g/100 ml) she felt better during the following ten days than during the preceding blood transfusion period. This is even more intriguing in view of the fact that several of her coagulation factors, i.e. prothrombin + proconvertin (factors II + VII) factor V factor IX and fibrinogen as well as the platelet count, were low On the 12th of Dec. she was given 300 ml of washed red cells as well as 500 ml of whole blood. On the following day she once more got nausea, abdominal pain, lowered blood pressure and vomited blood. Her hemoglobin

Table I Different coagulation factors in the patient's plasma

	21.11.60	25.11.60	29.11.60	2.12.60	28.11.61	Normal value	Methods
Coagulation time	8'13	5'35"	6'47"	5'49"	7'10" 9'45"	4 – 8'	Modified Hedenius (10)
In silicone tubes	20'	14 – 18'	12'–17'	10'–14	12'–26'	20'–30'	Modified Lee & White (15)
Bleeding time	2'30"	3'16"	–	2'26"	–	1 – 3	Duke (7)
Platelets/µl	83,000	56,000	48,000	45,000	230,000	200,000–400,000	Kristensson (12)
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Factor IX (%)	51	46	54	62	86	60 – 140	Nilsson et al (19)
Circ. anticoagulants	0	0	0	0	0	0	Prolong. of recalcif. time of normal plasma (19)
Fibrinogen g/100 ml in %	0.18	0.19	0.25	0.34	0.44	0.28 ± 0.06	Bergström et al. (3)
Fibrinolysis (µg/hr) at pH 6.3 at pH 7.1	100 5	0 0	0 0	0 0	0 0	31 ± 25 18 ± 34	Bergström et al. (3)

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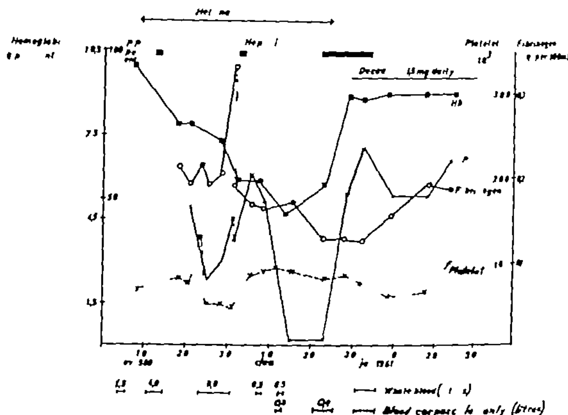


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blood transfusion therapy as well as during heparin therapy The results are shown in tables I and II and in fig. I

Coagulation analyses on November 8th and 18th had shown a shortened coagulation time in siliconized tubes and lowered platelet count. More thorough coagulation analyses on Nov 21st and later revealed that not only was the coagulation time somewhat shortened and the platelet counts lowered but the prothrombin + proconvertin, factor V and factor IX activity as well as the fibrinogen level were low No fibrinolysis or circulating anticoagulants were observed. An increase in apparent AHF (factor VIII) measured in a recalcification system using hemophilia A plasma as a substrate, was observed.

During whole blood administration an increase in prothrombin + proconvertin activity and fibrinogen level occurred an increase which on December 2nd was followed by a rapid decrease of the level of several coagulation factors and a clinical manifestation of shock. In a period when the patient's condition was rather good the pattern of the various coagulation factors was quite different (table II, 28.11.61) Thus the platelet count and the prothrombin + proconvertin activity were normal. The factor V activity was still somewhat low and the apparent AHF activity high.

Discussion

Bleeding episodes are often seen in liver cirrhosis These are mostly varicose bleedings but sometimes diffuse bleedings occur which may not be of this type. Cirrhosis of the liver is often accompanied by deficiencies in coagulation factors, especially prothrombin and proconvertin (3 20) A high frequency of hypofibrinogenemia and increased fibrinolytic activity is also found (8, 9 13 20) The high fibrinolytic activity often noted might well be the cause of the diffuse bleedings. However it has not always been possible to correlate the bleedings to increased fibrinolytic activity or to varicose veins. In the present case of advanced liver cirrhosis, there was no evidence of fibrinolytic activity or varicose veins as the cause of the hemorrhagic tendency Coagulation factor analyses revealed that regular changes occurred during the bleeding episodes. Low platelets counts, a decrease in fibrinogen, prothrombin (factor II) + proconvertin (factor VII) factor V factor IX, as well as an increase in apparent antihemophilic factor (factor VIII) activity was seen. This increase in factor VIII activity might be due to accumulation of intermediary coagulation products in the blood The pattern of multiple deficiencies in coagulation factors is very similar to that found after intravenous injections of thromboplastic material (22 28) during anaphylactic shock in rabbits (6, 12) and in the Sanarelli-Shwartzman reaction in animals and man (23 26) and also in obstetric bleeding complications (25)

Intravascular coagulation with consumption of several coagulation factors as a possible explanation of fibrinogenopenia in various clinical conditions of th type described above has been discussed by several authors (3,25) Several data as well as clinical observations suggest that intravascular coagulation might be responsible for the bleeding tendency in the patient with liver cirrhosis studied here. A rapid decrease in fibrinogen and prothrombin + proconvertin concentration was noted in connection with the bleeding episodes, a finding in good accordance with that reported by Bergström et al

Table II *Variations in coagulation time, platelet numbers, prothrombin + proconvertin activity, fibrinogen concentration and melaena during periods of hospitalization*

Date	Coagulation time	Coagulation time in silicone	Platelets per µl	PXP % (prothrombin + proconvertin)	Fibrinogen (g/100 ml)	Melaena
8. 11 60	3 19	13 15	65 000			
18. 11 60	2'00"	12	90,000		0.22	++
21 11 60	2'38	12 45	85,000	47	0.19	+++
23. 11 60	—	—		—	0.23	+++
24 11 60	—	—	130,000	—	—	—
25. 11 60	4 42	16 15	56,000	22	0.19	+++
28. 11 60	2'35	14'32	50,000	29	0.21	+++
2 12. 60	3 45"	12'35	45,000	44	0.34	+++
				36	0.19	
5. 12 60	2'35		85,000	59	0.17	+++
8. 12 60	2'30		90,000	50	0.16	+++
12. 12. 60			95,000			
15. 12. 60	2'30		90 000	2	0.18	+++
23. 12. 60	2'29	18'20	80 000	2	0.13	—
28. 12. 60	2 55	⁷3 10	85,000	54	0.14	+
2. 1 61	2 45		75 000	66	0.13	—
9. 1 61	4'00	22 10'	60,000	52	0.16	—
17 1 61	2'20		65,000	52	0.19	—
24 1 61	4'23	20'05	100,000	61	0.18	—
3. 5. 61	1 45	14 12	170,000	70	0.96	—
12 5. 61	2'25		280,000	75	0.48	—
7 6. 61			125 000			—
8. 6. 61	2'05	12 50	175,000	85	0.54	—
12. 6. 61	3 15	20 15	165,000	92		
15. 6. 61	3 40'		145,000	74	0.44	—
19 6. 61	2 12		120,000			—
24 8. 61	2 50	12'30	150,000	56	0.52	
3. 11 61			70,000	54	0.52	
28. 11 61	45	13'20	230,000	60	0.44	
7 3. 62	45	18 45	80,000	70	0.46	
Normal values	4—8	20—30	200 000—400 000	85—110	0.26 ± 0.06	

for three days the melaena ceased. She had had melaena between Nov. 16th and Dec. 28th. A four-month period of dexamethason therapy (1.5 mg daily) was started on Dec. 29th. She left the hospital in quite good condition on Jan. ?8th 1961.

The patient is still alive (July 3) and has remained in fairly good condition. In Dec. 1962 a stone was excised from the right ureter without complications. It is interesting to note that during dexamethason therapy (1.5 g daily) her paper electrophoresis became normal, her values of GPT decreased from 88 to 12 units and her prothrombin-proconvertin values became normal.

Results

Changes in hemoglobin values, platelet counts and coagulation factors were studied during several of the periods of

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During a period of marked hypocoagulability, low platelet count and decreased levels of prothrombin + proconvertin and fibrinogen, the patient felt remarkably well. When coagulation factors were administered through whole blood therapy she became ill, whereas transfusion of washed erythrocytes had no adverse effect. This may depend on an increased consumption of the coagulation factors after administration of the whole blood. Previously Bergström et al. (3) observed that the utilization of fibrinogen in their case with liver cirrhosis was higher when fibrinogen together with plasma was given than when fibrinogen only was administered.

During the heparin therapy in this patient the melaena disappeared, the hemoglobin content increased and the prothrombin + proconvertin activity and fibrinogen content of blood increased.

The decrease in the plasma coagulation factors was in the present case accompanied by a marked decrease in the platelet counts. Such a thrombocytopenia was also reported in the case of Bergström et al. (3). Probably a platelet disintegration is involved in the initiation of the intravascular coagulation. The cause of such a platelet disintegration in liver cirrhosis cases remains unknown. In the present case the low platelet values — around 40 000 per μl — may alone or in combination with the deficiencies in the coagulation factors have contributed to the bleeding tendency. Another possibility is that microthrombi in the capillaries might be the cause of the diffuse bleeding tendency.

Heparin effectively inhibits all phases in blood coagulation and would thus be an efficient remedy for this particular condition. Both successful and unsuccessful treatment with heparin of thrombotic thrombocytopenic purpura have been reported (1, 2, 4, 9, 16). One case of a Sanarelli-Shwartzman reaction in a woman with an obstetric complication has also successfully been treated with heparin (23). Also in anaphylactic shock in rabbits a protective action of heparin has been reported (11, 14, 24) with inhibition of the decreases in platelet count, prothrombin + proconvertin activity and fibrinogen which accompany this reaction (12).

Summary

An account is given of a case of cirrhosis of the liver successfully treated with heparin during two periods of acute bleeding and shock. Coagulation analyses showed a shortened coagulation time in siliconized tubes, lowered platelet count, low levels of prothrombin activity (factor II) + proconvertin (factor VII), factor V, factor IX and fibrinogen. No fibrinolysis or circulating anticoagulants were observed. An increase in apparent AHF (factor VIII) was observed. During a clinical manifestation of shock a rapid decrease of the level of several coagulation factors was noticed.

Several data as well as clinical observations suggested that intravascular coagulation might be responsible for the multiple deficiencies of coagulation factors and the bleeding tendency in the present patient with liver cirrhosis.

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Acta Medica Scandinavica. Vol. 175 fasc. 2, 1964

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Treatment of Acute Cases of Salmonella Infection and Salmonella Carriers with Ampicillin and Neomycin

By

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In Salmonella infection there is little chance of preventing the development of, or of curing, convalescent carriers with the aid of drugs. The chances of success in the treatment of chronic carriers are also slight. The best results have been achieved with G-penicillin-sodium in large doses (3, 4 8) But this therapy too, fails in a large number of cases. In addition, allergic complications are frequent.

A new form of penicillin, i. e. ampicillin was described by Rolinson and Stevens (9) in 1961 It is characterized by a broad spectrum and has a strong effect on many Gram-negative bacteria. According to Rolinson and Stevens, all strains of the Salmonella group under study were inhibited by a concentration which seldom exceeded 2.5 mcg/ml. As a rule, tetracyclin, G-penicillin-sodium and chloramphenicol have been effective only in higher concentrations. These observations have been confirmed by Stewart et al (10) among others. In contrast to tetracyclin and chloramphenicol, which only have bacteriostatic effect, ampicillin, like other penicillins, has a bactericidal effect.

In infections of the urinary tract due to Gram-negative bacteria, ampicillin has been used with success (1) It has also been suggested that this drug might be effective in infections of the alimentary tract caused by Gram-negative bacteria, in the first place Salmonella infections. But Stewart et al. (10) who treated 6 patients with salmonellosis typhi murium, failed to cure any of them. They administered 50—100 mg/kg of body weight daily for five days. Certain other workers, too have reported discouraging results in some cases (6 11 12)

Unfortunately ampicillin is susceptible to the effect of penicillinase, which may interfere with the effect of the drug in the intestine. If penicillin therapy were preceded by the administration of some substance which as far as possible eliminates the penicillinase-producing bacteria from the intestinal flora, the environment might become more favourable from the standpoint of the effect of ampicillin on bacteria of for instance, the Salmonella group. Neomycin is a substance possessing the properties required. Furthermore, it has been used with some success in Salmonella infections. Main (7) reported that he was able to cure 8 out of 9 chronic carriers by cholecystectomy in conjunction with neomycin treatment.

Submitted for publication July 30, 1963.

Table I Acute Salmonella infection

Salmonella strain	No. of cases	No. of negat. cases immediately after treatment	No. of negative cases within 2 months after treatment	No. of pat. excreting bacilli over 2 months after treatment
Group A Patients given combined neomycin-ampicillin therapy				
S. typhi murium	21	8	9	4
S. montevideo	2	—	1	1
S. [illegible]	1	—	1	—
S. haifa	1	—	1	—
S. enteritidis	1	1	—	—
S. paratyphi B	2	—	1	1
S. typhi	1	—	—	1
Total	29	9	13	7
Group B Patients given placebo tablets				
S. typhi murium	17	4	7	6
S. montevideo	3	—	—	3
S. anatum	1	—	1	—
S. paratyphi B	1	—	—	1
Total	22	4	8	10

Table II The effect of combined neomycin-ampicillin therapy in 10 convalescent and 3 chronic carrier

	Salmonella strain	No. of cases	No. of negativ. cases after treatment	No. of pat. excreting bacilli after treatment
Convalescent carriers	S. typhi murium	5	4	1
	S. paratyphi B	5	2	3
Chronic carriers	S. typhi murium	1	—	1
	S. paratyphi B	1	—	1
	S. montevideo	1		1
	Total	13	6	7

and a minimum of 8 but as a rule 10—16 samples of faeces have been negative. Ten patients continued to excrete bacilli for over 2 months. In one of these cases the roentgenological finding from the biliary tract was pathological, and bacilli were present in the duodenal fluid.

No allergic reactions were observed during the therapy. Many patients developed diarrhoea, which soon stopped when the treatment was discontinued. Some patients complained of abdominal pain.

Convalescent carriers and chronic cases

As is seen in table II, 6 out of 10 convalescent carriers ceased excreting bacilli immediately after discontinuation of the

Since the beginning of 1962 the effect of combined neomycin ampicillin Doctacillin® (Astra Sweden) therapy on the excretion of bacteria in chronic and convalescent carriers and in acute cases has been studied at the Fourth Department of the Aurora Hospital Helsinki. Another group of acute cases simultaneously given placebo tablets served as controls.

Material and methods

Acute cases

These patients were divided into two groups, A and B. The patients admitted to the hospital on odd dates formed group A, those admitted on even dates group B.

Group A consisted of 29 patients, 21 women and 8 men aged between 17 and 69 years. The mean age was 36 years. They were given a combined neomycin-ampicillin therapy by mouth. The therapy was instituted not sooner than 3 days after the fever had subsided and the diarrhoea had abated Neomycin was administered for 4 days, initially 1 g 4 times at one hour intervals and thereafter 1 g at 6-hour intervals. The ampicillin therapy was instituted on the third day of treatment, and the patients were given 1 g at 6-hour intervals for 7 days.

Group B, serving as controls, comprised 22 patients, 13 women and 9 men, aged between 18 and 85 years. The mean age was 46 years. They received 2 placebo tablets 3 times a day for 7 days.

As a rule, the patients were hospitalized for 4—6 weeks. Samples of faeces were collected for bacteriological examination twice a week throughout the period of hospitalization, and subsequently twice a month, and after 3 months once a month. The period of observation varied between 6 and 10 months.

Convalescent and chronic carriers

This group consisted of 13 patients, 9 women and 4 men. Of these 10 were convalescent carriers who had excreted bacilli for less than one year The patients were given neomycin as described above. Immediately after the neomycin therapy was completed, ampicillin medication was instituted, 2 g at 6-hour intervals for 10 days. Samples of faeces were bacteriologically examined in the same way as in the foregoing group. The time of observation varied between 8 and 14 months.

Furthermore, a 32 year-old man, who had excreted bacilli only in the urine for over ? months, was treated with ampicillin alone. This patient had epididymitis also.

Determinations of the sensitivity of the Salmonella bacteria to ampicillin, G-penicillin-sodium and neomycin were performed by Ericsson's (2) disc method. The discs used were made by Ericsson and delivered in Finland by Messrs. Distra.

The strains of Salmonella are shown in tables I and II

Results

Acute cases

The results of the combined neomycin-ampicillin therapy in group A are shown in table I About one third or 9 out of 29 patients, ceased excreting bacilli immediately after the treatment They have been regularly checked for 6—10 months, and from each patient a minimum of 8, but as a rule 12—16 negative samples of faeces have been obtained Furthermore, 13 patients ceased excreting bacilli within 2 months, after the discontinuation of treatment. They have been checked for 6—10 months and a minimum of 8 but as a rule 10—15 samples of faeces have been negative. Seven patients continued excreting bacilli for more than 2 months after the therapy was completed In 2 of these cases cholesterosis was observed by cholecystography In no case was a culture of the duodenal fluid positive.

Table I shows the results in the control group B which was given placebo tablets Four out of 22 patients, or about one fifth, ceased excreting bacilli immediately after discontinuation of the sham treatment. Within 2 months another 8 ceased excreting bacilli The patients have been checked for between 6 and 10 months,

The results of the tests for resistance to ampicillin, G-penicillin-sodium and neomycin are shown in table III. As is seen group A includes 2 cases in which an ampicillin concentration of 64 mcg/ml or more was required to inhibit growth of the Salmonella bacteria. Otherwise no difference in sensitivity was observed. The concentration of G-penicillin-sodium required to inhibit bacterial growth was as a rule two or three times higher.

Discussion

The fact that ampicillin has a markedly inhibiting effect on various Salmonella bacteria *in vitro* seemed to give hopes that with the aid of this drug the development of convalescent carriers of Salmonella might be prevented and chronic carriers perhaps cured. The first reports on treatment of Salmonella infections with ampicillin have not been encouraging however (6, 10, 11). In pilot investigations performed by the present authors, too, ampicillin alone was ineffective in the treatment of Salmonella carriers. Since it could be assumed that penicillinase-producing bacteria might have a disturbing influence on the effect of ampicillin, this drug was given in conjunction with an initial neomycin therapy in the hope that the bacteria in question would thus be largely eliminated from the intestinal canal.

Unfortunately the combined neomycin-ampicillin therapy did not answer the expectations. There was no convincing difference between the group given neomycin-ampicillin and the control group given placebo tablets. In the treated group the number of patients who ceased excreting bacilli was somewhat larger than in the control group, but the series is too small to allow of definite conclusions. The difference may be due to chance. Considering that 6 out of 10 treated convalescent carriers ceased excreting bacilli, it may perhaps, be stated that combined neomycin-ampicillin therapy occasionally yields a good result just as treatment with G-penicillin-sodium in massive doses has been found to do (3, 4, 8). It does not seem probable that it was due to chance that all of the 6 patients mentioned above ceased excreting bacilli immediately after the therapy, even if it may have been so in some of them.

It is obvious, however, that the usual criteria of a cure, i. e. 5—10 negative samples of faeces collected at 2- or 3-day intervals and not earlier than one week after the discontinuation of treatment, are not adequate. In the present study 5 to 7 negative samples — in one case even 9 negative samples — were obtained after the therapy had been completed in cases where subsequent samples were again positive. This shows that the population of bacilli may be so much weakened during the treatment that negative samples are obtained, and it is therefore necessary to check the patients for many months before they are regarded as cured.

In the case of urinary carriers ampicillin therapy seems to be worth a trial. The male urinary carrier included in the present series, who was treated with ampicillin alone, ceased excreting bacilli after the therapy. Several investigators have reported that during ampicillin treatment a high concentration of this substance was noted in the urine (1, 5, 10). According to Knudsen and Rolinson (5) about 30 per cent of the dose administered is excreted in the urine.

The tests for resistance showed that the Salmonella bacteria were equally sensitive to ampicillin in the two groups of patients, i. e. patients treated with ampicillin and patients given placebo

Table III Sensitivity of Salmonella to ampicillin, neomycin and G-penicillin sodium as determined by the disc method of Ericsson

Group A = patients treated with neomycin and ampicillin.
Group B = patients treated with placebo tablets.

Pat. groups	No. of pat.	*Ampicillin* Minimum inhibiting concentration (mcg/ml)								
		0.5	1.0	1.5	2	3	6	9	64	100
A	24	1	—	8	7	2	4	—	1	1
B	15	—	1	7	2	2	—	1	—	—
		Neomycin Minimum inhibiting concentration (mcg/ml)								
		2	5	8	16	25	50	100		
A	24	1	5	3	6	6	2	2		
B	15	1	1	2	3	2	3	1		
		G-penicillin-sodium Minimum inhibiting concentration (mcg/ml)								
		1.8	2.4	3.6	4.8	6.0	7.2	7.6	>100	
	47	5	4	14	6	4	2	6	6	

combined neomycin-ampicillin therapy Of these, 2 had been operated upon for biliary disease prior to the treatment. Cultures from the duodenal fluid had been positive. After operation the excretion of bacilli had continued In 2 cases roentgenological examination revealed a normal gall bladder In one case biliary dyskinesia was observed but no bacilli were found in the duodenal fluid One patient had gallstones and cultures from the duodenal fluid were positive but owing to her poor general condition no operation could be performed After the neomycin ampicillin therapy the excretion of bacilli ceased, however In this group the period of observation was from 12 to 14 months and the average number of negative samples obtained was 14 Four patients continued to excrete bacilli after the treatment Of these, 3 had been operated upon for biliary disease prior to the therapy Cultures from the duodenal fluid had been positive. One patient had positive duodenal cultures and gallstones, but could not be operated upon owing to severe cardiovascular disease.

None of the 3 chronic carriers treated ceased excreting bacilli In one of these cases cholecystectomy had been performed This patient had had positive duodenal cultures. One patient had gallstones and positive duodenal cultures, but could not be operated upon owing to old age and poorness of the general condition The third patient had a normal gall bladder and negative duodenal cultures.

The patient (male) who had excreted bacilli only in the urine was treated with ampicillin alone. He ceased excreting bacilli immediately after the therapy and cultures have been negative.

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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The Detection of Significant Bacteriuria

By

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Pyelonephritis is increasingly recognized as a serious disease, which often leads to grave complications after varying duration (12, 24). In more than half the afflicted cases it causes death, and it is the most common single cause of death of uremia (18).

Infection may not be the sole cause of the progressive destruction of renal tissue in pyelonephritis (6), though little doubt exists that chronic or recurrent infection plays a major part in the development of the disease. Therefore the detection of active infection is of vital importance.

Bacterial growth from a urine specimen does not necessarily mean active infection, since contamination is apt to occur either during collection or due to the normal urethral flora (4). On the other hand active infection may be present without any symptoms and signs except bacteriuria (3, 15, 19). Such infections may easily remain unrecognized, and it is well known from autopsy studies that many cases of pyelonephritis were unsuspected during life (13, 18).

Quantitative bacteriological methods as suggested by Marple (15) are helpful in the detection of significant bacteriuria. When active infection is present, the bacterial count exceeds 100,000 bacteria per ml of freshly obtained urine, whereas the number of organisms per ml usually remains below 10 000 when it is a matter of contamination alone (8, 9, 11, 14, 19, 20). Even if voided midstream samples are used, contamination may as a rule be distinguished from true infection (17). By this technique the upper limit of definite contamination may be taken as 50,000 bacteria per ml (16). A bacterial count of 50—100 000 calls for further investigation, but counts of this magnitude are found only in a few per cent of the cases (10).

As a routine procedure quantitative bacterial counting may often be found too intricate and time-consuming. A simple and reliable chemical method for the detection of significant bacteriuria (defined as greater than 100 000 bacteria/ml freshly obtained urine) would be useful for routine screening of all patients.

Submitted for publication August 1, 1963.

tablets In 2 cases only in group A 64 mcg/ml or more were required to inhibit bacterial growth. These patients were among those who ceased excreting bacilli within 2 months after the discontinuation of therapy In the majority of cases bacterial growth was inhibited by a concentration of ampicillin of 3 mcg/ml or less. The concentration of G-penicillin sodium required was usually 2 to 3 times higher Moreover no difference in the sensitivity to neomycin was observed between the two groups

Many patients developed diarrhoea and abdominal pain during the treatment. These side-effects were particularly marked in some cases in the group which was given 2 g of ampicillin every 6 hours. Allergic complications did not occur

Summary

The authors studied the effect of combined neomycin ampicillin therapy on the faecal excretion of bacilli in 29 acute cases of Salmonella infection while placebo tablets were simultaneously given in 22 acute cases serving as controls

No convincing difference was observable between the two groups although the number of patients who ceased excreting bacilli was somewhat higher in the treated group

Furthermore, 10 convalescent carriers and 3 chronic carriers were treated with neomycin-ampicillin Six convalescent carriers ceased excreting bacilli. It seems, therefore, that this therapy may sometimes be successful.

It is obvious that the patients have to be checked for a long period of time before they are regarded as cured It appears that the population of bacilli in the intestine may be so much weakened by the treatment that a series of negative samples may be obtained although subsequent samples are positive.

One patient who excreted bacilli only in the urine ceased excreting them after the ampicillin treatment.

Tests for resistance performed *in vitro* showed that the Salmonella bacteria were inhibited by an ampicillin concentration of 3 mcg/ml or less, except in a few cases.

A number of patients developed transient diarrhoea and abdominal pain, which were rather severe in some cases. No other side-effects occurred.

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Addendum

During the course of printing this paper W E. Bullock (Amer J Med. Sci. *246* 76 1963) has reported that 3 out of 7 carriers of Salmonella treated with ampicillin ceased excreting bacilli. W P U Kennedy A. T Wallace & J McC. Murdoch (Brit. Med. J *II* 962, 1963) treated 7 carriers of Salmonella and 3 patients with acute Salmonella infection with ampicillin. Five patients ceased excreting bacilli.

Table II. Correlation of bacterial counts and TTC tests

Bacterial count	TTC pos.	TTC neg.	Total
$>10^5$	67	7 (9.5%)	74
$<10^5$	6 (4.7%)	125	131

Table III. Correlation of bacterial counts and TTC tests. Proteus-infected specimens are excluded

Bacterial count	TTC pos.	TTC neg.	Total
$>10^5$	61	6 (9.0%)	67
$<10^5$	5 (4.0%)	124	129

Table IV. The influence of the duration of incubation upon the occurrence of false negative and false positive TTC tests

Time (hrs)	Bacterial count (bact/ml)	Total	TTC neg.	TTC pos.	% ± 1 SE
4	$>10^5$	74	14		18.9 ± 4.5
	$<10^5$	131		1	0.8 ± 0.8
6	$>10^5$	74	11		14.9 ± 4.1
	$<10^5$	130		3	2.3 ± 1.3
8	$>10^5$	72	5		6.9 ± 3.0
	$<10^5$	65		5	7.7 ± 3.3

Sediment. To get an impression of the relationship between pyuria and bacteriuria ordinary microscopy of the sediment was performed. The urines are grouped according to the number of leucocytes per high-power field. The urines were centrifuged for 4 minutes at 1,500 r.p.m.

Results

The results of the TTC test as compared with quantitative bacterial counting are given in table II. Proteus species swarming on the plates make the counting difficult. Exclusion of the 9 proteus cases, however, does not change the results significantly (table III). Of the 67 specimens, where both the bacterial counting and the TTC test were positive, only 41 (61.2 %) gave a positive Griess test. Two positive Griess tests occurred among 125 uninfected urines. Smears from 63 infected urine specimens were Gram stained. In no more than 30 (47.6 %) was the result of oil-immersion microscopy recorded as positive. A positive result was recorded in 8 of 110 uninfected urines.

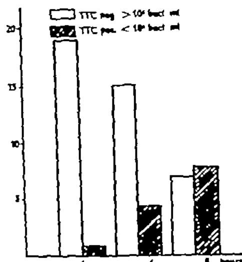


Fig. 1. Dependence of the TTC test on the duration of incubation. White columns show the percentage of false negative TTC tests, shaded columns show the percentage of false positive TTC tests. Apparently the best time of reading is after 8 hours of incubation.

A pilot study made it clear that the number of positive TTC tests increased with longer duration of incubation. 137 of the samples were examined after 4, 6 and 8 hours, 67 after 4 and 6 hours. The influence of time is illustrated in table IV and fig. 1. The percentage of "false negative" TTC tests decreases, while the percentage of "false positive"

Table I. The diagnoses in the 166 patients

Pyelonephritis chronica primaria	29
Pyelonephritis chronica secundaria	22
Pyelonephritis acuta	4
Recurring pyuria	7
Hypertension, normal renal function	8
Hypertension, impaired renal function	6
Diabetes mellitus, no renal lesion	9
Proteinuria	6
Fever of unknown origin	7
Miscellaneous	68
Total	166

It is the purpose of this paper to evaluate a new chemical test and to take into consideration some already known simple tests for urinary tract infection.

The new test depends upon the ability of bacteria to reduce triphenyltetrazolium chloride (TTC) to red insoluble formazan. This compound has antiseptic properties. However, bacteria commonly found in urinary tract infections as well as the common contaminants are but little sensitive, and the reduction takes place in any concentration allowing growth at all (25). Simmons and Williams (21) in a study of 480 urine specimens found a close correlation between a TTC test and quantitative bacterial counting. Smith and Schmidt (22) found a related compound alphanaphtyltetrazolium to be unreliable in testing urine for significant bacteriuria.

Material and methods

A total of 205 urine specimens from 166 patients were examined. No treatment with antibiotics was given prior to the collection of the samples. It was our intention to get about half the specimens from patients with active infection, but the patients were not otherwise selected. The diagnoses are stated in table I.

The specimens were collected as midstream-voided first morning urine. The urethral orifice was cleansed with benzalkonium chloride before the collection of urine in sterile containers. The collection was carried out by specially instructed nurses. A few specimens were from patients with an indwelling catheter.

The urines were examined by quantitative bacterial counting, microscopy of sediment, microscopy of Gram-stained smear of uncentrifuged urine, Griess nitrite test and the TTC test.

Culture was done using calibrated loops as described by Hoeprich (5). The loops used in this study had been previously checked against serial dilutions of urines by one of the authors (M.D.). Culture was made on blood-agar containing 1 % glucose and 1 % peptone. Discrete colonies were counted up to a limit of 100 000 col./ml (100 colonies on the plate), higher figures being designated > 10^5. Part of each specimen was sent to the routine bacteriologic laboratory for identification and for a sensitivity test to the commonly used antibiotics.

TTC test. The reduction takes place at an alkaline pH. Stock-solution was prepared by dissolving 750 mg triphenyltetrazolium chloride in saturated disodium hydrogen phosphate. The solution was sterilized by Seitz filtration. Working solution was prepared by diluting 4 ml of the stock-solution with 96 ml Na_2HPO_4. The test was carried out by mixing 0.5 ml of the working solution and 2.0 ml of urine in a test tube. The test-tubes were then incubated at 37° C and examined after 4 h and 8 hours. A positive reaction is indicated by a red precipitate. Generally the reaction is fairly easy to read. Highly concentrated or coloured urines may impede the reading; in such cases the precipitate, which is insoluble, may be washed with water. TTC at concentration used in the tubes does not hamper the growth of organisms usually encountered in urine specimens (25).

Griess nitrite test was included in our study as a comparison because of its great simplicity even if it is known to fail in about 30 % of infected urines. The technique indicated by Smith et al. was used (1, 7, 22, 23).

Gram stain. One loopful of uncentrifuged urine was allowed to dry on a glass slide. After Gram staining, a positive result was recorded when microorganisms were readily seen by oil-immersion microscopy.

failed to detect pathogenic organisms, and that with small numbers of contaminants a positive TTC reaction occurred only twice in 125 cases.

The occurrence of the various bacterial species does not differ essentially from other authors findings (2) In 41 of the infected urines the organism was E. coli, whereas this organism occurred as a contaminant in 10 cases. In 131 cases the bacterial count was below 10^4 46 of these urines were sterile, and in 58 the organism was staphylococcus albus. No special measures were taken against the Gram-positive contaminants because we were interested in the ability of the TTC test to distinguish between contamination and true infection. In the 4 cases where staphylococcus albus was present in a number exceeding 10^5/ml, the TTC test was negative.

A bacterial count between 50,000 and 100,000 was found in 4 cases (1.9 %) In all other cases there was no difficulty in determining whether significant bacteriuria was present or not. The use of voided urine (midstream samples) may be impossible in old people, who cannot cooperate, but is otherwise a satisfactory sampling technique.

The TTC test seems fairly reliable as compared with the more accurate bacteriologic method. In combination with a routine qualitative bacteriologic examination a correct answer may be expected in about 97 %. The test is recommended wherever quantitative bacteriologic methods are not readily available

Summary

Two hundred and five urine specimens were examined by quantitative culture, two chemical tests, microscopy of sediment and Gram-stained smears. Significant bacteriuria was found in 74 cases, 67 of which gave a positive reaction with triphenyltetrazolium chloride (TTC) after 8 hours of incubation at 37° C. The Griess test failed to detect about 40 % of the infected urines, Gram stain proved still more inaccurate. The TTC test is almost as accurate as quantitative bacterial counting and may be expected to give correct answers in 97 % when combined with routine qualitative bacteriologic examination. The test may be useful in screening large number of patients and wherever quantitative bacterial counting is not easily performed

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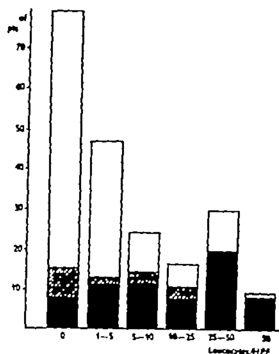


Fig 2 The count of leucocytes per high power field, 205 urine pecimens.
Black number of specimens where both the bacterial counts and the TTC tests were positive (active infection) Shaded One of these tests positi e (probably active nfection) Whit Both tests negati (no infection)

reactions increases on prolongation of the incubation time. After 8 hours, the per centages are equal

In 125 urine specimens both the bac terial count and the TTC test were nega tive. Pyuria was present in 16 of those urines. On the other hand pyuria was absent in 17 of the 67 urines with signi ficant bacteriuria and a positive TTC test. The findings are illustrated in fig 2.

Discussion

A good agreement was found between the bacterical counts and the TTC tests the results were in accordance in 93 7 % Both tests were positive in about 36 % 88 urines were collected from patients with known urinary tract disease. In 51 of those significant bacteriuria was found. 112 urines were from patients with no evidence of urinary tract disorders. In no less than 23 (20 6 %) cases significant bacteriuria was found. This finding emphasizes the well-known risk of over looking symptomless urinary tract infection (9 15)

The Griess test failed to detect c. 40 % of the infected urines, but a positive Griess test strongly suggests infection. The Gram stain proved in our hands quite inadequate as an indicator of significant bacteriuria

Most of the patients with marked pyuria had significant bacteriuria as well. However 25 % of the cases with significant bacteriuria had no pyuria (less than 5 leucocytes per high power field)

Thus, among the simple laboratory tests here evaluated, only the TTC test seems to be of sufficient accuracy in detecting significant bacteriuria. Simmons and Williams (21) obtained a high degree of correlation between the TTC test and the bacterial counting after 4 hours of incubation In this study the best results were obtained after 8 hours. As the percentage of "false positive TTC reactions increases rapidly from 6 to 8 hours, the incubation period should not be further prolonged

Six "false positive TTC tests occurred. In 4 of those cases the bacterial count was fairly high (10—50 000 bact /ml) and the organisms were coli and proteus. In the remaining 2 cases few colonies of staphy lococcus albus were found The latter was also the case in 4 of the 7 urines with "false negative TTC test In the remaining 3 cases the organisms were E. coli, proteus and an unidentified Gram neg rod Thus it seems justified to state that in only 2 (or 3) cases out of 74 has the TTC test

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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Pulmonary Emphysema and Alpha$_1$ Antitrypsin Deficiency

By

Sten Eriksson[1]

A previously unknown type of dysproteinaemia with a marked reduction of one of the main components of the electrophoretic α_1-fraction, α_1-antitrypsin, was described in an earlier paper (12). This abnormality was often found to be associated with severe, early pulmonary emphysema.

This paper is concerned with laboratory and clinical studies of familial α_1 antitrypsin deficiency and emphysema.

Material

The family lives in the district of Eksjo, Småland. The pedigree is seen in fig. 1. Clinical and laboratory data on the propositus (II in fig. 1 case 3 in case reports) have been given in brief previously (12). A brother (II in fig. 1 case 1 in case reports) was treated at his local hospital for emphysema. Clinical data on this patient were obtained from the archives of that hospital and from his relatives. The patient died at home in 1954, i. e. before the present investigation was started. This explains the lack of electrophoretic data. The propositus (II) and his sister (II in fig. 1 case 2 in case reports) were repeatedly admitted to the department of chest diseases of their local hospital (Eksjö) and in January 1963 to the Department of Medicine, Malmö General Hospital. Of the remaining family members still living, 14 were examined, the examination including electrophoresis and determination of the α_1-antitrypsin. Thirteen of these 14 denied any symptoms of pulmonary disease, while the 14th, a 5-year-old girl, (IV$_4$) had had symptoms of asthma for 2 years. She had never been in hospital. As to the propositus' parents, both of whom are dead, only meagre data are available. The father (I) who had lived to an advanced age, died in uraemia; the mother (I$_2$) from miliary tuberculosis. Neither of the parents had had asthma. The propositus' paternal aunt had had asthma and had died at home from a vascular insult. Three paternal uncles of the propositus denied any symptoms of pulmonary disease. None of the maternal relatives of the propositus are known to have had pulmonary disease. Two of the propositus' sibs died in childhood: one (II$_3$) was a girl, who died at 9 years from diphtheria and the other a boy (II$_4$) who died at 2 months from malformation, probably spina bifida. II$_5$, a man, born in 1931 is mentally retarded (Down's syndrome) and is being cared for in hospital. He has no pulmonary disease.

Submitted for publication August 6, 1963.

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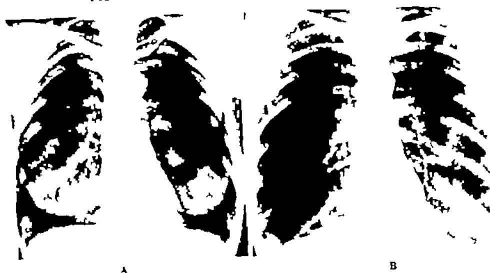


Fig. 2. Chest X-ray A case 2 (1963), B case 3 (1963)

could be influenced by bronchodilatator drugs, aminophylline was given intravenously in dose of 250 mg and the spirometry repated after 15 minutes.

PaO was measured according to polarographic method. *PaCO* was measured according to Astrup et al. (1)

Case reports

Case 1 (II$_1$) A painter born in 1909. Apart from diphtheria during childhood he had always felt well and he had done his military service. I 1945 when he was 36, he noticed incipient dyspnoea on exertion. In 1947 to 1949 this symptom progressed with acute exacerbations in association with infections of the respiratory tract, especially *during* the winter months. Infections were often accompanied by attacks of asthma with wheezing and productive cough but his main complaint was dyspnoea on effort. He had no known history of allergy H was admitted to his local hospital in 1949 where examination revealed emaciation and restricted chest movement, but no cyanosis and no signs of cardiac incompensation. Physical examination of the chest yielded weak respiratory sounds on the bases. No abnormal sounds could be heard. Cardiac dullness was found to be absent and the sounds were weak. B. P 130/90 mmHg. Chest X-ray showed severe, generalized emphysema. The heart appeared as typically drop-shaped. ECG showed a slight inversion of T_1. The ESR, the WBC and RBC and the number of eosinophils were normal. There was no proteinuria or glycosuria. Various cutaneous tests for allergy were negative. After temporary improvement the patient was pensioned in 1952 because of ventilatory insufficiency

H was re-admitted in June 1953 because of acute respiratory tract infection. Chest X-ray showed marked emphysema with flat and low diaphragma, increased hilar shadows and increased translucency of the lung fields. The cardiac silhouette was narrow and vertical. N bullous cysts could be seen. The *electrocardiographic recording* showed tachycardia, negative P_I and T_I, large and pointed P waves and pathological ST in CR_4. He had lost 6 kg body-weight since 1949. Hb and RBC were normal and there was no eosinophilia.

During the following year the disease progressed rapidly with disabling dyspnoea, frequent infections with symptoms of asthma, and marked deterioration of his general condition. The patient died at home in 1954. He was not examined post mortem.

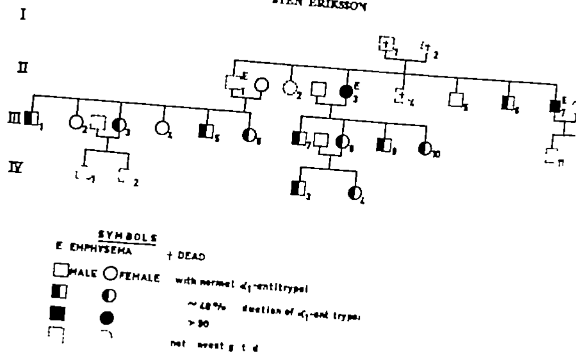


Fig 1 The pedigree. II is the propositus.

Methods

Quantitative paper electrophoresis was performed with a calcium lactate containing barbital buffer according to Laurell et al. (13) and *agar gel electrophoresis* in 1 % agar (purified) was run on cooled (+ 4 C) glass plates (4× 40 cm) with the same buffer a modified Wieme (16) procedure

Antitryptic activity was determined by a modification of the method of Erlanger et al. (8) for estimation of tryptic activity with BAPA (benzoyl D L-arginine p-nitroanilide hydrochloride) as substrate. The electrophoretic distribution of the antitryptic activity in sera was determined after separation in agar (see above) The gel was cut into 1 cm segments. The slices were eluted, by agitation overnight, in 0 05 M barbital buffer pH 8 6, at + 5 C.

α_1-antitrypsin" was calculated from the difference between total antitryptic activity and the antitryptic activity found in the α_1-fraction after electrophoresis in agar gel, The values are expressed as μg trypsin inhibited by 1 ml serum.

Sodium in sweat was determined according to Gibson and Cooke (9)

Pulmonary function tests

The terminology used is mainly that suggested by Pappenheimer (14)

The abbreviations used are listed below:

VC	Vital capacity
$FEV_{1.0}$	Forced expiratory volume in 1 sec.
$FEV_{1.0}\%$	$\frac{FEV_{1.0}}{VC}$
FRC	Functional residual capacity
TLC	Total lung capacity
PaO	Arterial oxygen tension
$PaCO_2$	Arterial carbon dioxide tension
LCI	Lung clearance index according to Becklake (2)
$FEV_{1.0}$	was determined with a Bernstein-spirometer as modified by Berglund et al. (3) The normal values used for $FEV_{1.0}$, $FEV_{1.0}\%$ and VC are those given by these authors.
FRC	was measured with the open circuit technique of Darling et al. (6) as modified by Bouhuys et al. (4) The normal values are taken from Grimby and Söderholm (10)

The distribution of inhaled gas in the lungs was judged from the lung clearance index according to Becklake (2) i. e. from the volume of oxygen required to wash out N_2 (down to 2 %) from 1 l of lung volume The normal values for LCI have been obtained from Bouhuys (5)

In order to see to what extent the obstructive pulmonary disease found in the patients

vertical, clockwise-rotated heart without signs of hypertrophy There was no evidence of cor pulmonale.

The results of spirometric examination are given in table I.

Laboratory studies including determination of Hb, RBC and WBC, total number of eosinophils, bromsulphalein retention, GOT GPT culture of sputum, albustix and clinistix tests on the urine, determination of the sodium content of sweat and electrophoresis of the urine revealed nothing pathological.

Case 3 (II) A painter born in 1925. He smokes about 10 cigarettes day He had done his military service. In 1944 he had been admitted to his local hospital because of pneumonia. I 1954 and 1957 he was ex amined at the same hospital because of stabbing pain in the chest in association with deep breathing Chest X-ray on those oc casions revealed nothing remarkable, and the diaphragmatic mobility was good. In 1959 he had dyspnoea and feeling of tension in the chest on slight exertion. These symptoms steadily progressed and afterwards became worse in association with infections of the upper respiratory tract, which the patient often had, particularly during the autumn and winter months. During remissions he rarely had cough and then with only scanty and transparent expectorate. His dominating symptom was dyspnoea on effort. In Dec. 1961 he had right-sided pneumonia. Chest X-ray showed emphysema. His functional residual capacity was 6.9 l, M.B.C. was 25 l/ min. and FEV 35 % of VC. There was no eosinophilia. Treatment with breathing exercises and bronchodilytic drugs produced some improvement, but during 1962 his working capacity was impaired. He had constant dyspnoea and he could only walk about 50 m t time. Since the onset of the disease he had lost been 10 kg. including 3–4 kg during 1962.

The patient was admitted to Malmö General Hospital in January 1963 for check-up.

On admission examination revealed emaciation, slight resting dyspnoea and sligh cyanosis, but no clubbing of the fingers. Cardiac dullness was absent, and the heart sounds were feeble. B.P 120/80 mm Hg The chest was barrel-shaped with increased sagittal diameter and showed indrawings of the lower intercostal spaces and the use of accessory muscles. The diaphragm was low there was hyperresonance and the breath-sounds were faint. N abnormal sounds were audible Chest X-ray (fig. 2 B) showed emphysema with low flat diaphragm having


Fig. 3. Paper electrophoretic pattern of serum in case 3 (below), compared with the of normal serum (above)

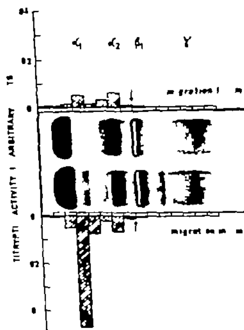


Fig 4 Partition f serum antitryptic activity after separation in agar gel. Serum from case 3 (bove) compared with normal serum (below) Arrows indicate points of application

Table I Pulmonary function in cases 2 and 3 (For abbreviations see text. Figures within brackets are predicted normal values)

	Case 2 Sex female, Height 161 cm, Weight 50 kg, Age 48 yrs		Case 3 Sex male, Height 171 cm, Weight 50 kg, Age 38 yrs	
	Before amino-phylline	After amino-phylline	Before amino-phylline	After amino-phylline
VC (l)	3.8 (3.0)	3.3	2.9 (4.4)	3.3
$FEV_{1.0}$ (l)	1.0 (2.4)	1.0	0.6 (3.6)	0.7
$FEV_{1.0}$ (%)	40 (79)	40	25 (78)	25
FRC (l)	4.1 (2.3)	4.4	6.4 (3.9)	6.0
TLC (l)	6.5 (4.2)	6.6	8.5 (6.5)	8.2
LCI (l)	15.0 (<10)	21.2	>15 (<10)	>15
Pa_{O_2} (mm Hg)	81 (83–104)	—	81 (83–104)	—
Pa_{CO_2} (mm Hg)	34 (34–43)	—	37 (34–43)	—

Case 2 (II_2) A woman born in 1915. The patient is a smoker with a daily consumption of 3—4 cigarettes. At the age of 17 she had obstinate bronchitis. In 1953 she was subjected to subtotal bilateral strumectomy because of thyrotoxicosis. Chest X-ray that year showed a somewhat flat diaphragm. Since 1953 she had noticed increasing dyspnoea on exertion with wheezing and a feeling of tension in the chest, especially in cold weather. The attacks were ushered in by coughing with copious production of expectorate. She also noticed increased breathlessness on exposure to certain synthetic detergents. From 1956 on she had had obstinate infections with fever, productive cough and dyspnoea during the winter months. During 1959 she had more or less constant dyspnoea with wheezing and cough and scanty production of mucoid expectorate. Examination at the chest department of her local hospital in 1960 revealed no cardiac incompensation, increased resonance to percussion and prolonged expiration. Rhonchi were heard over large areas of the lungs. Chest X-ray showed a flat diaphragm and a narrow vertical heart. No eosinophils were found in the sputum, and the number of eosinophils in the blood was not increased. In December 1960 she was re-admitted because of asthma. Inhalation tests revealed hypersensitivity to dust, feathers, dog- and cat epithelium. Desensitization treatment for her allergy was started but was not completed owing to bouts of fever with increasing bronchospasm, cough and mucopurulent expectoration. She was treated with broncholytic drugs and anti-catarrh vaccines and periodically with steroids. In June 1961 she had a right sided bronchopneumonia.

Treatment of her allergy was resumed in 1962 since when her condition has somewhat improved and infections have been less frequent. Her shortness of breath has persisted, but she can manage a walk on level ground fairly well, she coughs but little and produces only scanty expectorate.

In January 1963 the patient was admitted to Malmö General Hospital for a check-up.

On admission examination revealed no cyanosis, oedema or clubbing of fingers. Mild resting dyspnoea and restricted chest movement were noted. The chest was barrel-shaped with increased sagittal diameter and increased subcostal angle. Cardiac dullness was absent and the heart sounds were distant. B.P. 120/90 mm Hg. Chest X-ray (fig. 2A) showed emphysema with flattening of the diaphragm and small respiratory excursions, which were measured as 1 cm on the right side and 2 cm on the left. Hilar shadows were prominent. The heart was narrow and vertical. No bullous cysts were seen. ECG showed a

normal mean value. A comparison between the values for the electrophoretic α_1-globulins and the values for α_1-antitrypsin shows a poor correlation. From fig 5 it is evident that the family members with respect to their α_1-antitrypsin activity fell into 3 groups, namely (A) normal amount, (B) about 60 % of normal and (C) less than 10 % of normal. The various α_1-antitrypsin levels are also given in fig 1 to illustrate the pattern of inheritance.

Of the married partners of members of the family only three have so far been studied. The concentration of α_1-antitrypsin was normal in all three.

The concentration of α_1-antitrypsin was also determined in two cases of manifest mucoviscidosis and was found to be normal in both.

Discussion

That the α_1-antitrypsin deficiency was familial in the generations studied is clear from figs. 1 and 5 The members of the family belong largely to three groups according to the serum α_1-antitrypsin levels, namely normal, 60 % of normal and less than 10 % of normal. It may be assumed that 60 % α_1-antitrypsin represents a heterozygous state, and less than 10 % a homozygous state of a genetic defect transmitted by an abnormal recessively inherited gene. Since the α_1-antitrypsin concentration in I and I_2 in fig 1 is not known it is not possible to conclude with certainty that the inheritance was recessive, but the fact that II (case 2) who seems to be an affected homozygote married to an unaffected man, has four heterozygous children, strongly favours the assumption of a recessive mode of inheritance. The occurrence of unaffected persons, slightly affected heterozygotes, and affected homozygotes in the second generation suggests that both I_1 and I were heterozygotes. II (case 1) was married to an unaffected woman. Four of their children are heterozygotes and two are normals, this indicating that their father was a heterozygote. In several other families (to be reported later) with α_1-antitrypsin deficiency and so far only partly investigated we have found that the members belong to the same three groups in respect of the α_1-antitrypsin concentration.

The occurrence of emphysema in three siblings lends support to the hypothesis that genetic factors might be of importance in the pathogenesis of this disorder Serious pulmonary diseases in which the clinical picture was dominated by emphysema were seen in 9 of 14 cases of pronounced α_1-antitrypsin deficiency This frequent combination of emphysema and α_1-antitrypsin deficiency can hardly be due to chance. Since the biological significance of α_1-antitrypsin is not yet known, any attempt to explain the causal mechanism of emphysema in patients with antitrypsin deficiency cannot be more than guesswork. In a recent discussion of the pathogenesis of pulmonary emphysema Ebert and Pierce (7) pointed out that the occurrence of the disease in younger individuals might be related to genetic factors resulting in weakness of the fibrous framework of the lung Seebohm and Bedell (15) presented a compilation of cases with primary emphysema in which dyspnoea on exertion was the initial and dominating symptom, while Hurst (11) gave a review of the literature on familial occurrence of emphysema. These two types described by Seebohm and Bedell and by Hurst appear to be rare compared with the overall frequency of pulmonary emphysema. Moreover no

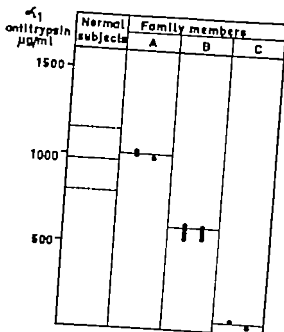


Fig 5 The family members grouped with respect to α_1 antitrypsin concentration
A normal α_1 antitrypsin concentration,
B about 60 % of normal,
C less than 10 % of normal

Table II Paper-electrophoretic α_1-values (g/100 ml) and enzymatically determined α_1-antitrypsin (μg/ml) in the family members

Family member	α_1	α_1-antitrypsin
II_2 (Case 2)	0.15	61
II_5	0.28	1,012
II_6	0.31	610
II_7 (Case 3)	0.15	47
III_1	0.30	545
III_2	0.38	995
III_3	0.28	570
III_4	0.35	1,028
III_5	0.28	567
III_6	0.30	600
III_7	0.29	590
III	0.29	585
III	0.38	630
III	0.38	660
IV_3	0.28	547
IV_4	0.30	557
Normal values M ± 2 SD	0.23–0.38	795–1 153

a mobility range of 2 cm The heart was narrow and vertical. No bullous cysts were seen.

ECG showed a vertical, clockwise rotated heart without signs of hypertrophy The P waves in lead II were large and pointed. There was no evidence of cor pulmonale

The results of spirometry are given in table I

The same laboratory investigations as in case 2 were performed and revealed nothing pathological.

Results

Serum electrophoresis and concentration of α_1-antitrypsin

The paper electrophoretic pattern of serum in case 3 is presented together with a normal serum in fig 3 The absence of a distinct α_1 band is clearly demonstrated In fig 4 the protein pattern and distribution of antitryptic activity in serum from case 3 after electrophoresis in agar gel are demonstrated with a normal serum for comparison. The reduction in antitryptic activity in the α_1-region is clearly demonstrated The antitryptic activity in the α_2-region is of about the same amount as in the normal serum.

The results of serum α_1-antitrypsin determination and paper electrophoretic determination of the α_1-globulins in the family members are shown in table II Subnormal α -globulin values were obtained in cases 2 and 3 Among the other family members the electrophoretic α values fell within the normal range. The results of the determination of the α_1 antitryptic activity revealed less than 10 / of the normal amount in cases 2 and 3 Of the other 14 family members studied only three (II III_4 and III) had normal values The majority of the family members showed a reduction of their α antitrypsin activity to just above half the

normal mean value. A comparison between the values for the electrophoretic α_1-globulins and the values for α-antitrypsin shows a poor correlation. From fig. 5 it is evident that the family members with respect to their α_1-antitrypsin activity fell into 3 groups, namely (A) normal amount, (B) about 60 % of normal and (C) less than 10 % of normal. The various α_1-antitrypsin levels are also given in fig. 1 to illustrate the pattern of inheritance.

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analyses of the electrophoretic patterns in these two forms are available.

Another observation arguing for the significance of genetic factors in the pathogenesis of emphysema in the three siblings is the early and almost equal age of onset (35 years) of the symptoms. Most cases of emphysema occur much later in life. In the two males (cases 1 and 3) the clinical picture was dominated by dyspnoea on exertion. The absence of previous bronchitis or other chronic diseases of the lung argues for the emphysema being of the primary or essential type In the two males the disease appeared to progress rapidly with associated loss of body-weight. No eosinophilia could be demonstrated In the female the clinical picture was dominated by symptoms of asthma and hypersensitivity to various exogenous agents but without eosinophilia The course of the disease appears to have been less serious

The results of spirometry (table I) were largely the same in case 2 and 3 though pulmonary function was impaired more in case 3 in whom the clinical findings were also more accentuated. The $FEV_{1.0}$ and % $FEV_{1.0}$ were considerably reduced The distribution of the inhaled gas was very uneven as shown by the L.C.I Even the resting PaO_2 was slightly decreased in cases 2 and 3 The severe impairment of pulmonary function found in these studies was of the obstructive type seen in asthma and emphysema. The fact that aminophylline did not significantly improve pulmonary function is suggestive of irreversible changes in the lungs e. g changes known to occur in emphysema

In the absence of reversible, acute hyperinflation of the lungs the roentgenograms showing flattened and low diaphragms, prominent hilar shadows and vertical narrow heart silhouettes may be thought to indicate emphysema.

The absence of eosinophilia in the present cases was also noteworthy Liver function appeared to be normal and electrophoretic analysis of the urine showed no increased amount of α_1-antitrypsin so that renal losses could not be held responsible for the low concentration of the α antitrypsin in the serum.

The sodium content of the sweat in the cases in which it was determined was normal. There was no laboratory or clinical evidence for mucoviscidosis in the family studied.

Summary

α_1 antitrypsin deficiency in the serum and pulmonary emphysema were observed in various members of one family Three levels of α_1-antitrypsin concentration were found namely normal, about 60 % of normal and less than 10 % of normal. Observations made argue for a recessive type of heredity In three siblings pulmonary emphysema occurred early in life. In two of these the α_1-antitrypsin concentration was less than 10 % of normal and in the third no determination could be made as the patient was already dead.

Acknowledgments

The author is indebted to Doc. S. E. Lindell at the Department of Clinical Physiology Malmö, for advice and facilities put t his disposal in performing the pulmonary function tests.

The technical assistance of Miss Eva Nilsson is greatefully acknowledged.

This investigation was supported by grants from Albert Österlunds Stiftelse Malmö, and from Department f Health, Education and Welfare Bethesda, Maryland, U.S.A. No. E-4703.

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In the absence of reversible, acute hyperinflation of the lungs, the roentgenograms showing flattened and low diaphragms, prominent hilar shadows and vertical narrow heart silhouettes may be thought to indicate emphysema.

The absence of eosinophilia in the present cases was also noteworthy Liver function appeared to be normal and electrophoretic analysis of the urine showed no increased amount of α_1-antitrypsin so that renal losses could not be held responsible for the low concentration of the α_1-antitrypsin in the serum.

The sodium content of the sweat in the cases in which it was determined was normal There was no laboratory or clinical evidence for mucoviscidosis in the family studied

Summary

α_1 antitrypsin deficiency in the serum and pulmonary emphysema were observed in various members of one family Three levels of α_1 antitrypsin concentration were found namely normal, about 60 % of normal and less than 10 % of normal Observations made argue for a recessive type of heredity In three siblings pulmonary emphysema occurred early in life. In two of these the α_1 antitrypsin concentration was less than 10 % of normal and in the third no determination could be made as the patient was already dead.

Acknowledgments

The author is indebted to Doc. S. E. Lindell at the Department of Clinical Physiology Malmö, for advice and facilities put at his disposal in performing the pulmonary function tests.

The technical assistance of Miss Eva Nilsson is greatefully acknowledged.

This investigation was supported by grants from Albert Österlunds Stiftelse, Malmö, and from Department of Health, Education and Welfare Bethesda, Maryland, U.S.A. No. E-4703

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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Hyperlipemia

A Report of an Unusual Case Complicated by Bone-lesions, Macrocytic Anaemia and Leukemoid Bone Marrow

By

Einar Wolff Sørensen

Lipidosis comprises an etiologically heterogeneous group of diseases which are characterised by the common feature of lipid accumulation in the mesenchymal tissues. The accumulated lipids may have an extremely varied chemical composition.

The lipidoses may be classified in various ways. They embrace the following conditions

1 Xanthomatosis.
2. Hyperlipemia.
3. Schuller-Christian s disease.
4 Gaucher' disease.
5 Niemann-Pick s disease.
6. Amaurotic family idiocy (Tay Sach s disease)
7 Gargoylism.

Each of the conditions mentioned has its own, relatively distinct, characteristic signs and symptoms.

This communication is particularly concerned with group 2, the hyperlipemias. Hyperlipemia is not found in the other lipidoses. The distinction is made between a primary essential hyperlipemia and a secondary form in which the hyperlipemia is a factor in another recognised disease, e. g diabetes mellitus, acute pancreatitis, chronic pancreatitis, lipid nephrosis and van Gierke s disease.

The most important signs and symptoms in essential hyperlipemia are shown in table I

As is evident, neither th skeletal system nor the hematopoietic system are affected in this condition.

Case report

The patient is man born in 1907 His parents were not related to each other Both parents and their brothers and sisters, 15 persons in all, attained an advanced age. They were of normal stature and enjoyed good health. The patient has 6 brothers and sisters (table II). One brother and one sister were delicate from birth and died before one year The cause of death is unknown. One of the patient' brothers is mentally ill (manic depressive psychosis) and another brother is blind, having had weak sight from birth. H

Submitted for publication August 9 1963.

Table III Results of blood examinations

Hb	14.8 g/100 ml
RBC	3.8 mill./mm³
WBC.	4,600/mm³
Thrombocytes	380,000/mm³
Reticulocytes	3–7‰
MCV	99 mμl.
MCH	42 pg
MCHC	42%
ESR	22 mm/h
Bleeding time	6 min
Coagulation time	5 min
Vitamin B_{12}	672 pg
Serum proteins	
Albumin	3.4 g%
Globulin α_1	0.3 g%
Globulin α_2	1.1 g%
Globulin β_1	1.1 g%
Globulin γ	1.3 g%
Fibrinogen	0.25 g%
Glucose tolerance test	Normal
Plasma iron	239 γ/100 ml
Blood type	0 RH +
Prothrombin time	110%
Osmotic fragility	Normal
Ca in serum	11.4–13 mg%
P in serum	3.5–3.9 mg%
Creatinine in serum	0.5 mg%
Urea in serum	40 mg%
Blood sugar fasting	110 mg
Thymol turbidity test	2.9 units
Phosphatase alk.	2 units
Lactic dehydrogenase	65 units
Differential counting of leukocytes	
Juvenile neutrophils	3
Eosinophils	1
Segmented neutrophils	5
Lymphocytes	90
Cold agglutinins	Not found

considered suitable for military service. In 196_ his chest was X-rayed because of his bronchitis. This showed a tumor-like shadow corresponding to the 3rd left rib anteriorly. On examining previous X-rays from 1930, it was seen that the patient had the same tumor-like shadow at this time.

Apart from bronchitis the patient has been quite healthy and has been in heavy work as a builder labourer. He has normal appetite and has not noticed any polyuria or polydipsia.

Table IV Examination of urine and feces

Urine	
Specific gravity	1.020
Albumin	Negative
Bence-Jones protein	Negative
Glucose	Negative
Blood	Negative
Ca	126 mg/24 hrs
P	701 mg/24 hrs
17-ketogenic steroids	6.8 mg/24 hrs
Amino acids	Normal content
5-HIAA	3.6 mg/24 hrs
Diastase	Normal content
Feces	
Color	Normal
Consistency	Normal
Daily vol.	Normal
Test for blood	Negative
Fat content.	11.8 g/24 hrs

Clinical findings

The patient looks well and has a good color. No pigmentation of the skin or mucous membranes. No pingueculae. No palpable lymph-nodes. No cyanosis.

Height 160 cm. Weight 47 kg. B. P. 125/85. Pulse regular 72/min.

Tongue appears normal. A few bronchitic adventitious sounds heard over both lungs. Liver and spleen not palpable.

Testes of normal size and consistency. Spinal fluid normal. W. R. -ve. Tuberculin test -ve. Histamine-fast achlorhydria demonstrated.

Psychiatric investigation revealed nothing pathological.

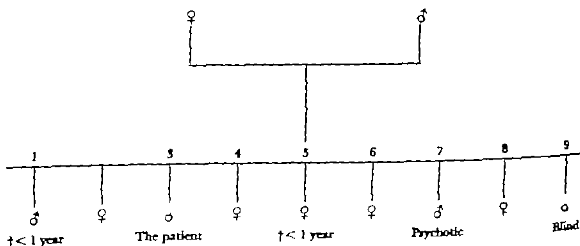
The results of blood, urine and feces examinations are shown in tables III and IV. There is a macrocytic anemia with distinctly raised hemoglobin concentration in the red cells. The serum calcium is slightly raised. Serum protein electrophoresis shows increased α_2-globulin (about twice the normal amount). Fecal fat is slightly raised. The rest of the investigations showed no obvious departure from the normal.

The fundus of the eye was photographed (fig. 1). The vessels appear as whitish streaks such as is generally seen in hyperlipemia.

Table I The symptoms and signs of idiopathic hyperlipemia

	Juvenile form	Adult form
Etiology	Unknown	Unknown
Inheritance	Not constant	—
Sex	—	—
Nature of lipemia	Neutral fat (milky serum)	Neutral fat (milky serum)
Hepatomegaly	Not unusual	Unusual
Splenomegaly	Not unusual	Unusual
Eruptive xanthomata	Present	Not always present
Jaundice	—	—
Electrophoretic patterns of serum proteins	Elevated α_1 α_2 and β-globulins	Elevated α_1, α_2 and β_1-globulins
Disturbance of carbohydrate metabolism	—	Slight elevation of blood sugar and tendency to glycosuria
Bone lesions	—	—
Hematopoetic disturbances	—	—
Mental disturbances	—	—
Heparin-clearing of the milky serum	Slight/moderate and temporary	Slight/moderate and temporary

Table II The members of the patient's family



The patient has no children. Three of his sisters and one brother have children and they are all normal.

is also rather backward mentally. Of the patient's brothers and sisters, nos. 4, 6, 7 and 8 have been investigated. They are of normal height and weight and there is no evidence of disease.

From childhood, the patient has been bothered by bronchitis with cough and scanty sputum. In recent years, he has been increasingly breathless on exertion but has otherwise been healthy.

When under treatment for an injury to his right leg in 1949 it was noted that he had milk-white serum. At 21 years of age he was 148 cm tall and was, therefore, not



Fig. 2. The sternal marrow



Fig. 3. X-ray of the chest



Fig. 4. X-ray of the lumbar spine.

proximal metaphyses of the long bones, particularly in the humerus, femur and tibia, there is totally irregular bone structure characterised by intersecting, thick and thin, sclerotic bands with irregular zones of clearing in between. I the shoulder girdle and pelvic bones, there is network of ir regular clear patches bounded by thickened, sclerotic septa. I the vertebral column, fine longitudinal streaking is seen in the corpora but no significant osteoporosis. No compression or destruction. There is nothing remarkable in the distal ends of the long bones nor in the small bones of the hands and feet. The structure of the cranium suggests fine vacuolation. The ribs are expanded and retort-shaped towards the costo-chondral junction. This is most pronounced in the case of the 3rd left rib. The bone structure in the ribs appears rather rarefied but there is no demonstrable destructive change.

Histological and histochemical investigation of the bone tissue from the ilium (P. B. Lenow



Fig 1 The fundus of the eye.

The lipid content of the patient's serum is shown in table V There is a considerable increase of neutral fat and free fatty acids but the content of phospholipid and cholesterol is not materially increased It appears that when the patient has a low fat diet, the content of neutral fat and free fatty acids is somewhat reduced but the concentration remains markedly raised. When the lipid investigation is carried out after incubation of the serum at 37° C for 12 hours, the phospholipid content is found to be reduced to about 50 whilst the amounts of neutral fat, free fatty acids and cholesterol are unchanged. The lipid content, determined before and after heparin administration, shows a reduction of neutral fat and free fatty acids to 463 mg

The serum lipid in 4 of the patient's brothers and sisters has been determined. Table VI shows the individual values. Each of these is seen to lie within normal limits (sister no 4 was not fasting when the blood specimen was taken)

The sternal marrow (fig 2) gives an impression of great activity Erythropoesis is lively and normoblastic. A few megaloblasts are seen. Myelopoiesis is very lively and completely dominating in some areas. Cells representing all stages of maturation are seen but in some areas, blast cells predominate as in acute leukemia. Abundant fat is present in the marrow and isolated "foam cells (lipid macrophages) are seen which have a certain similarity to Gaucher's cells. The peripheral blood shows macrocytosis and hyperchromasia but is otherwise completely normal.

X-ray investigation of the skeletal system (Prof. Hobæk) (figs. 3—8) shows that in the

Table V Lipids in the patient s serum. All values in mg%

	Total lipids	Phospho-lipids	Neutral fat + fatty acids	Cholesterol
On normal diet	5,320	302	4 740	478
On low-fat diet	3 740	397	2,937	406
On low fat diet after incubation.	3,515	191	2,918	406
On fat-free diet	2,870	253	2,249	278
On fat-free diet after heparin	2,295	185	1 786	324
On low-fat diet after discharge from hospital	4,395	481	3,333	481

Table VI Lipids in serum from the members of the patient family (cf table II) All values in mg%

No. in family chart	Total lipids	Phospholipids	Neutral fat + fatty acids	Cholesterol
2	980	247	435	298
4	1 125	237	624	264
8	960	243	477	240
7	910	139	537	234

Morbid Anatomy Department, Gades Institute) shows (figs. 9 and 10)

In the frozen section, lamellated bone tissue is seen and bone marrow containing bundant, Sudan-stained lipid, lying intracellularly In these cells, large numbers of doubly refractile needles are visible in polarised light. Staining with thionine-tartaric acid, according to Feyrter method, shows no evidence of metachromatic lipid. In the paraffin-embedded material, bone fragment is seen with regular lamellated bone spicules. There is no evidence of any osteoclastic activity In the bone marrow large groups of lipid macrophages predominate, which have pale finely granular cytoplasm and small, round eccentric nuclei. These cells are relatively large Occasionally very long, cleft-shaped spaces of the type seen in cholesterol deposition are found between the cells. Apart from this, there are many round spaces *without visible content* and w th diameter 5—6 times that of the lipid-containing cells. The P A S. reaction in the lipid-containing cells is negative. The reaction to iron is negative. Morphologically this seems to be matter of deposition of neutral fat, partly cholesterol. Eosinophilia and giant cells are entirely missing. There is certain morphological similarity to osseous xanthomatosis such as may be seen in Schuller-Christian disease, but in that disease there is no hyperlipemia Morphologically and histochemically Gaucher's disease is unlikely The morphological diagnosis is osseous lipidosis with deposition of neutral lipids and some cholesterol in the bone marrow

Diagnosis. Hyperlipemic osseous lipidosis.


Fig. 9 Biopsy from crista iliaca. The bone marrow is replaced by lipid macrophages. Hematoxylin-eosin × 40.


Fig 10 Same biopsy as fig 9 Between lipid *macrophages fat-containing round spaces* and cholesterol clefts are seen. Hematoxylin-eosin 160

Discussion

This is a lipidosis of the hyperlipemic type which, from the clinical history and find ngs, is of primary essential type. In dd tion t the symptomatology characteristic of this primary essential hyperlipemia, the patient described also has pronounced blood changes and peculiar bone lesions. The clinical picture is different from that which characterises the other lipidoses mentioned.

The *blood changes* reported here do not seem to ha e been described in any other lipidosis but, in rare cases of Schuller Christian disease, leukemic blood picture with a leukemic bone marrow reaction is described. A genuinely raised *hemoglobin concentration in* the red cells is seen in familial spherocytosis, but there is no evidence of this condition in the case described. It may be mentioned that the cause of familial spherocytosis is thought t depend either on the failure of the glycolytic mechanism of the red cells, or on the failure of their lipid



Fig 5 X-ray of the pelvis.



Fig 7 X ray of the left tibia



Fig 6. X-ray of the left humerus.



Fig 8. X ray of the left femur

| Anatomy Department, Gades Insti- sow. (figs. 9 and 10)
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Diagnosis Hyperlipemic osseous lipidosis.



Fig 9 Biopsy from crista iliaca. The bone marrow is replaced by lipid macrophages Hematoxylin-eosin 40.



Fig. 10. Same biopsy as fig 9 Between lipid macrophages fat-containing round spaces and cholesterol clefts are seen. Hematoxylin-eosin 160.

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Fig 7 X-ray of th left t bia.



Fig 6 X-ray of the left humerus.



Fig 8. X ray of the left femur

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the Department of Medicine, Karolinska Institutet at Serafimerlasarettet, Stockholm, Sweden

The Return to Work of Patients with Myocardial Infarction[1]

By

Gunnar Biörck and Eva M. Wedelin

In most developed countries myocardial infarction presents a medical problem of increasing importance. At the same time, the interest in rehabilitation procedures and social services for persons with limitations in physical fitness has increased. It is only natural, that cardiologists try to find out, what the problems of their infarct patients are, when the acute illness has subsided and convalescence and return to work have to be considered.

It is quite obvious that such problems are different for an active man in his forties and for the retired octogenarian. It is also obvious, that the problems are different in an affluent society with full employment together with well developed social security and pension schemes as compared with those in a developing country with great unemployment and no or limited social measures. Every study of the rehabilitation and employment situation of patients with myocardial infarction must take into consideration both these factors. The present study deals with city population in an affluent society with reasonably well developed social security including the old-age pension at the age of 67. The patient material represents an average hospital population of myocardial infarcts (1, 2, 9) with no particular bias with regard to sex, age or occupation.

In a previous study of this kind in Malmö, Sweden (3, 4) it was found that survivors of myocardial infarction in that town (the third largest in Sweden with 200 000 inhabitants) had a very favourable prognosis with regard to their return to work. This observation has been endorsed by studies from Göteborg (Sweden's second town, 350 000 inhabitants) where — in a younger infarct population with a mean age of 48 years — a similar rate of return to work was observed (8). On the other hand, representatives of the rehabilitation services in our country have asked why so relatively few cardiac patients are referred to them. Implying that cardiologists might not have realized to the full extent the needs of their patients in the light of recently available services

Submitted for publication August 9, 1963.

[1] Presented during the meeting of the WHO Expert Committee on Rehabilitation of Cardiacs in Geneva 22—29 July 1963.

metabolism (1) The aforementioned observations may perhaps support the latter theory Familial spherocytosis differs from other hereditary hemolytic anemias merely in the characteristic abnormality in the phospholipid-containing surface of the erythrocytes (1) Following incubation the erythrocytes in familial spherocytosis will lose more lipid than normal red cells (2 3) In the case described however there is a distinct *reduction* in the amount of phospholipid in the serum after incubation

Bone lesions are usually present in Schüller Christian's disease and in Gaucher's disease Radiologically the lesions seen in this case are quite different from those seen in the two conditions mentioned The morphological findings, as well as the histochemical deviate from the above mentioned lipidoses in this case.

Summary

A detailed description is given of a patient with hyperlipemia. The hyperlipemia, which mainly concerns neutral fat and fatty acids, is combined with quite uncommon bone lesions and hematological findings. It has not been possible in the literature to find a report of this constellation of symptoms. The patient therefore might represent a new syndrome.

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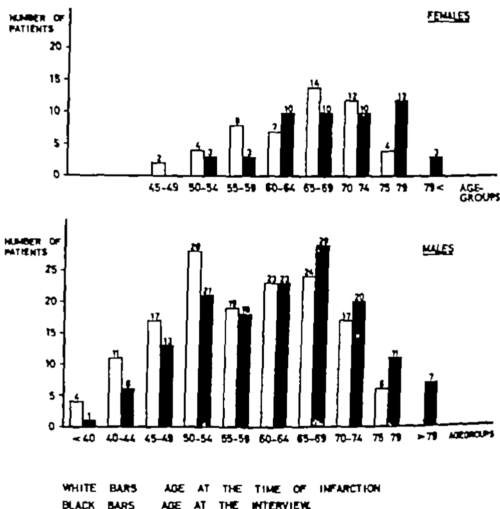


Fig 1 Study of the patients with regard to sex and age at the time of their last infarct and at the interview

(5) It therefore seemed warranted to reexamine the problem of the return to work of our patients with myocardial infarction in order to see what medicine and social services might further contribute to their health

Material and methods

An attempt was made to trace all patients who had been in hospital for myocardial infarction in the department of medicine Serafimerlasarettet, Stockholm during the years 1950–1961 There were 354 survivors belonging to the area of Greater Stockholm, to whom our study for practical reasons had to be restricted Of these 354 patients, 125 had died in the meantime, while 14 patients could not be traced and 15 for various reasons were unable or unwilling to participate The remaining 200 – ranging in age between 36 and 87 years – were all interviewed by a questionnaire either at the hospital or in their home In the case of inadequate medical supervision, provisions were also made to establish new contacts. The material was coded and subsequently handled by means of automatic data recording

Whereas little bias is introduced by the 29 patients who could either not be traced or were unwilling or unable to cooperate, the 125 patients who were dead present an as yet unsolved methodological problem, to which future efforts have to be devoted. In as much as the present study chiefly aims at a better knowledge of the needs after the hospital period of survivors of myocardial infarction, the em-

phasis was on the material content of the interview. If, on the other hand, the effects of early activity versus prolonged rest are to be compared judging from the end results, or the wisdom of returning to the same work be compared with retirement, one must find out whether the survivors and those who have died belong to the same population or present differences. This is obviously very difficult in retrospective study. However not until the retrospective study is made can prospective study be properly designed.

Results

The number of patients included in the study and their distribution with regard to sex and age, both at the time of their last *infarct* and at the interview are seen in fig. 1. In this study emphasis is on the *last* recorded infarct, for obvious reasons, whereas in purely descriptive materials, as in (1) and (9) the *first* infarct is recorded. The materials, therefore, are not strictly comparable.

There were 149 males and 51 females in the material which represents a predominance of males in comparison to the hospital population of infarcts in general (9). The original material of 354 patients, out of which these 200 infarcts are taken, was composed of 248 men and 106 women. Their mean ages at the last infarction were 61.1 and 66.9 years of age, whereas the corresponding figures in this material are 58.6 and 63.2. These figures *indicate, that* the patients who have died were several years older at the time of their infarct than those who survived and were available for interview. The mean ages for the 14 patients, who could not be traced were 61 for the 10 men and 71 for the 4 women. Corresponding figures for the 15 patients who were unable or unwilling to participate were 63.2 for the 13 men and 64.5 for the 2 women. Consequently both these groups are older than the group that came to interview. Despite this, they increase the male preponderance in the group of survivors.

The average follow up time for the men was 3.9 years and for the women 4.0 years. Of the 200 patients, 120 (60 %) — 113 men and 7 women — were working at the time of their last infarct, and of those 120 no less than 19 or 16 per cent, continued their work despite the fact that they were already enjoying their old age pension. The eighty patients, who were not working at the time of their infarct, consisted of 36 men and 44 women, with mean ages of 71.3 and 66 years, respectively. None of these patients had resumed any work after the infarction. The mean ages of those who were working at the time of the infarct was 54.6 years for the 113 men and 57.5 years for the 7 women, thus considerably less than the mean ages in the total group.

Of the 120 patients, who were working *at the time of the infarct,* 99 (83 %) returned to some type of work, as shown in table I. Of these, 67 or 56 per cent, returned to their previous occupation and 32, or 27 per cent, to some less demanding work, either in the same place of work (16 %) or in some other place (11 %). The number of women who returned to work is so small that it can be disregarded in the following discussion, which will deal chiefly with the males.

It should be observed, that the 17 per cent previously working, who did not return to any work consisted of 19 men and 2 women with mean ages, respectively of 56.4 and 62.5 years of age.

All these patients stated as reason for retirement that they were too tired to go on working. Some had tried for a short while and then given up. Others were

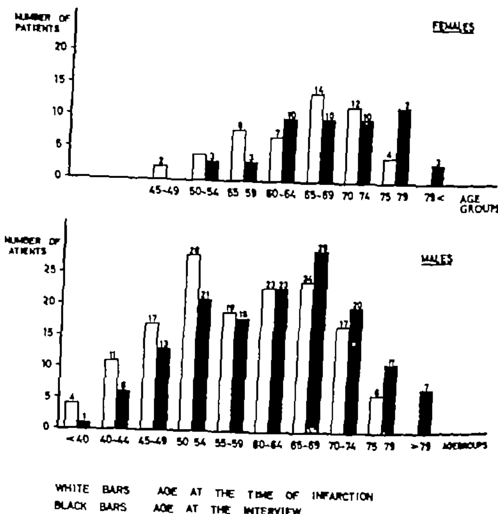


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All these patients stated as reason for retirement that they were too tired to go on working Some had tried for a short while and then given up Others were

Table I Return to work after infarction

Age at last infarction	Total no.	Not working before infarction	No change of work or working place	Change of work (to less demanding work) in same working place	Change of work (to less demanding work) in other working place	Not working after infarction
Males						
<40	4	–	2	–	2	–
40–44	11	–	7	1	3	–
45–49	17	–	10	2	1	4
50–54	28	–	16	6	2	4
55–59	19	–	11	3	2	3
60–64	23	–	9	4	2	8
65–69	24	15	9	–	–	15
70–74	17	15	1	1	–	15
75–79	6	6	–	–	–	6
	149	36	65	17	12	33
			94			
Females						
45–49	2	1	–	1	–	1
50–54	4	1	2	–	–	2
55–59	8	7	–	–	1	7
60–64	7	7	–	–	–	7
65–69	14	13	–	1	–	13
70–74	12	11	–	–	–	12
75–79	4	4	–	–	–	4
	51	44	2	2	1	46
			5			

still convalescent. In all cases, the lack of working capacity was medically certified and these patients, therefore, received "invalid pensions

A haemodynamic study is being performed in our department on a series of men 40—58 years old, with coronary heart disease, verified by past history electrocardiogram and coronary angiography (7) Whereas pressure-flow conditions at rest were not found to be significantly different from those in a control group of the same sex and age, the physical working capacity was found to be lower and changes in pressures and flow indicated impaired myocardial efficiency

The 19 men who did not return to work, with only one exception had some kind of manual work. Some were employed in very heavy work others were craftsmen on their own. This implies that they also all belonged to the middle and lower socio-economic groups.

Table II Return to work after infarction — in relation to number of infarcts

Age at last infarction	Return to same work				Less demanding work same working-place				Less demanding work other working-place				Not working after infarct			
	1 inf.	2 inf.	3 inf.	4 or more	1 inf.	2 inf.	3 inf.	4 or more	1 inf.	2 inf.	3 inf.	4 or more	1 inf.	2 inf.	3 inf.	4 or more
Males																
< 40	2	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
40–44	6	1	—	—	1	—	—	—	2	1	—	—	—	—	—	—
45–49	8	2	—	—	2	—	—	—	1	—	—	—	3	1	—	—
50–54	14	1	—	1	3	1	—	—	2	—	—	—	1	1	1	1
55–59	8	2	—	1	1	2	—	—	2	—	—	—	1	2	—	—
60–64	4	3	2	—	2	2	—		1	1	—	—	2	3	—	1
65–69	8	1	—	2	—	—	—	—	—	—	—	—	6	2	2	5
70–74	1	—	—	—	—	1	—		—	—	—	—	11	3	—	1
75–79	—	—	—	—	—	—	—	—	—	—	—	—	3	1	—	—
	49	10	2	4	11	6	—	—	10	2	—	—	29	15	3	8
	65				17				12				55			
Females																
45–49	—	—	—	—	—	1	—	—	—	—	—		—	1	—	—
50–54	2	—	—		—	—	—	—	—	—	—	—	1	—	—	—
55–59	—	—	—		—	—	—	—	1	—	—	—	5	2	—	—
60–64	—	—	—	—	—	—	—	—	—	—	—	—	5	1	—	1
65–69	—	—	—	—	1	—	—	—	—	—	—	—	8	3	2	1
70–74	—	—	—	—	—	—	—	—	—	—	—	—	9	3	—	—
75–79	—		—	—	—	—		—	—	—	—	—	3	1	—	—
	2	—	—	—	1	1	—	—	1	—	—	—	31	11	2	2
	2				2				1				46			

An analysis of the records of men below pension age, who did not return to work after infarction was made in order to ascertain, whether further attempts at rehabilitation might be tried. However it became evident, that this was feasible in very few cases only. The reasons for accepting a pension as the most adequate solution were in most cases age near pension age, the co-existence of several diseases, odd personality (mal-adjusted refugees and homespun "originals") *and the complete failure of social agencies to supply suitable apartments* (either on the ground floor or in a house with elevator). It therefore appears as if the question of adequate housing, preferably in the neighbourhood of a suitable place of work, represents a basic requirement in the rehabilitation of cardiac patients. Next in importance are probably attempts at a general psychological rehabilitation in those lacking in motivation to work or in adjustment to the work situation.

It is remarkable to note, that of 19 persons — 17 men and 2 women — who were enjoying old-age pension before the infarction 13 returned to their previous occupation, whereas 5 obtained a less demanding job and one — a woman —

Table III Return to work after infarction — in relation to marital status

Age at last infarction	Return to same work		Less demanding work – same working-place		Less demanding work – other working-place		Not working after infarction	
	Married	Single	Married	Single	Married	Single	Married	Single
Males								
<40	2	—	—	—	2	—	—	—
40—44	7	—	1	—	2	1	—	—
45—49	8	2	2	—	1	—	4	—
50—54	14	2	5	1	2	—	2	2
55—59	9	2	4	—	2	—	2	1
60—64	4	5	—	—	1	1	5	3
65—69	8	1	—	—	—	—	10	5
70—74	1	—	—	1	—	—	7	8
75—79	—	—	—	—	—	—	4	2
	53	12	15	2	10	2	34	21
	65		17		12		55	
Females								
45—49	—	—	—	1	—	—	1	—
50—54	1	1	—	—	—	—	1	—
55—59	—	—	—	—	1	—	5	2
60—64	—	—	—	—	—	—	5	2
65—69	—	—	1	—	—	—	6	8
70—74	—	—	—	—	—	—	4	8
75—79	—	—	—	—	—	—	1	3
	1	1	1	1	1	—	23	23
	2		2		1		46	

did not return to work. The type of work of these people before the infarct was sedentary in 5 and more or less physically active in 14 After the infarct 11 still continued in moderately active work

It should be noted that in some few instances the wife has felt compelled to seek employment because of the husband s illness. This pertains to five women with husbands in the age-group 50—54 years (at interview) two with husbands 55—59 years old and one woman in each of the husband age groups 60—64 65—69 and 70—74 years of age, making altogether 10 wives.

Of factors other than age, the following have been anlysed with regard to their influence on the return to work, viz. number of infarcts, marital status social group and type of occupation

It is of considerable interest that not less than 10 men were able to return to the same work after two infarcts and not less than 4 after four infarcts. No man below 45 years of age gave up working Of 99 men with one infarct 50 per cent returned to the same work and 20 per cent continued to work in a less demanding position whereas 30 per cent were not working

Table II. Return to work after infarction — in relation to social groups

Age at last infarction	Return to same work			Less demanding work same working-place			Less demanding work – other working-place			Not working after infarction		
	Upper soc. gr.	Middle soc. gr.	Lower soc. gr.	Upper soc. gr.	Middle soc. gr.	Lower soc. gr.	Upper soc. gr.	Middle soc. gr.	Lower soc. gr.	Upper soc. gr.	Middle soc. gr.	Lower soc. gr.
Males												
<40	—	—	2	—	—	—	1	—	1	—	—	—
40–44	6	—	1	1	—	—	1	1	1	—	—	—
45–49	2	7	1	1	1	1	—	1	—	—	2	2
50–54	4	10	2	—	2	4	2	—	—	—	2	2
55–59	5	3	1	—	1	2	—	2	—	—	1	2
60–64	4	2	3	—	1	3	—	1	1	1	5	2
65–69	2	7	—	—	—	—	—	—	—	—	13	2
70–74	—	1	—	1	—	—	—	—	—	2	8	3
75–79	—	—	—	—		—	—		—	1	5	—
	23	32	10	2	5	10	4	5	3	4	36	15
	65			17			12			55		
Female												
45–49	—	—	—	—	1	—	—	—	—	—	1	—
50–54	—	1	1	—	—	—	—	—	—	1	—	—
55–59	—	—	—	—	—	—	—	1	—	1		4
60–64	—	—	—	—	—	—	—	—	—	1	3	3
65–69		—	—	—	1	—	—	—	—	1	5	8
70–74		—	—	—	—	—	—	—	—	2	4	6
75–79		—	—	—	—	—	—	—	—	—	2	2
		1	1	—	2	—	—	1	—	6	17	23
	2			2			1			46		

There were no remarkable findings with regard to marital status. The apparently high number of single men not working is probably explained by the age distribution in this group. However it points to a particular need for social services within this group, as is — of course — also true for the elderly women.

Tables IV and V together show the good social prognosis of sedentary work (which is usually synonymous with middle or upper socio-economic groups). The fairly high prevalence of such patients in this material may indicate the validity of one of the following statements: 1) Sedentary workers are particularly liable to get infarcts. 2) Sedentary workers are particularly fortunate in their capacity as survivors of an infarct.

It is of a certain interest that the great majority of the patients have managed to arrange their re-employment directly with their employers. Less than 10 patients tried to use the official agencies for employment and rehabilitation and in only one case, a man 36 years of age with diabetes, was this approach successful.

Table V Return to work after infarction — in relation to kind of occupation

Age at last infarction	Return to same work			Less demanding work — same working-place			Less demanding work — other working place			Not working after infarction
	Sedentary occupat.	Moderate activity	Strenuous physical occupat.	Sedentary occupat.	Moderate activity	Strenuous physical occupat.	Sedentary occupat.	Moderate activity	Strenuous physical occupat.	
Males										
<40	—	1	1	—	—	—	1	1	—	—
40—44	3	2	—	1	—	—	1	2	—	—
45—49	3	7	—	1	1	—	—	1	—	4
50—54	6	7	3	3	3	—	2	—	—	4
55—59	5	5	1	2	1	—	—	1	1	3
60—64	5	3	1	1	3	—	1	1	—	8
65—69	3	5	1	—	—	—	—	—	—	15
70—74	—	1	—	1	—	—	—	—	—	13
75—79	—	—	—	—	—	—	—	—	—	6
	27	31	7	9	8	—	5	6	1	55
	65			17			12			
Females										
45—49	—	—	—	1	—	—	—	—	—	1
50—54	—	2	—	—	—	—	—	—	—	1
55—59	—	—	—	—	—	—	—	1	—	7
60—64	—	—	—	—	—	—	—	—	—	7
65—69	—	—	—	1	—	—	—	—	—	14
70—74	—	—	—	—	—	—	—	—	—	12
75—79	—	—	—	—	—	—	—	—	—	4
	—	2	—	2	—	—	—	1	—	46
	2			2			1			

In the decision as to the return to work, the subjective health conditions of the patients naturally play an important role It is quite remarkable that — as seen from table VI — 109 men regarded themselves as healthy and only 40 as not healthy whereas the opposite trend was observed in women — 17 healthy as against 34 not healthy The mean ages at interview were, respectively 62 6 and 62 years for the men and for the women 67.5 and 70 years of age.

No tendency was observed for "single" people to regard themselves as less healthy than married people. Altogether 15 men and 3 women were working although they did not consider themselves as "healthy"

This finding of a particularly impaired state of health in women is also substantiated by the judgement of their capacity for work at home (table VII) In the opinion of the interviewer the men were more apt to judge their capacity and state

Table VI

A. *Subjectively healthy — not healthy at the interview in relation to marital status*

Age at inter view	Males				Females			
	Healthy		Not healthy		Healthy		Not healthy	
	Married	Single	Married	Single	Married	Single	Married	Single
<40	1	—	—	—	—	—	—	—
40–44	5	—	1	—	—	—	—	—
45–49	6	1	6	—	—	—	—	—
50–54	16	1	3	1	—	1	1	1
55–59	12	2	4	—	3	—	—	—
60–64	10	7	5	1	2	1	4	3
65–69	13	6	8	2	1	1	5	3
70–74	9	6	4	1	2	2	3	3
75–79	4	4	1	2	1	2	4	5
80<	4	2	1	—	—	1	—	2
	80	29	33	7	9	8	17	17
	109		40		17		34	

B. *Subjectively healthy — not healthy at the interview in relation to working — not working after infarction*

	Males				Females			
	Working	Not working	Working	Not working	Working	Not working	Working	Not working
40	1	—	—	—	—	—	—	—
40–44	5	—	1	—	—	—	—	—
45–49	6	1	4	2	—	—	—	—
50–54	17	—	1	3	1	—	1	—
55–59	14		2	2	1	2	—	—
60–64	14	3	2	4	—	3	1	6
65–69	13	6	4	6	—	2	1	8
70–74	6	9	1	4	—	4	—	6
75–79	2	6	—	3	—	3	—	3
80	1	5	—	1	—	1	—	2
	79	30	15	25	2	13	3	31
	109		40		17		34	

of health objectively than were the women who seemed to underrate themsel es.

As already mentioned, one of the main objectives of this study was to examine the need of special rehabilitation programmes for infarct patients. The patients were asked, whether they had any opinion concerning the desirability and usefulness of organized rehabilitation after hospitalization.

Of the 200 patients interviewed 110— 73 men and 37 women — had no ideas or suggestions to give. Twenty-one — 18 men and 3 women — expressed interest in more ctive types of rehabilitation, including physical exercises. But 35 — 27

Table V Return to work after infarction — in relation to kind of occupation

Age at last infarction	Return to same work			Less demanding work — same working place			Less demanding work — other working place			Not working after infarction
	Sedentary occup.	Moderate activity	Strenuous physical occup.	Sedentary occup.	Moderate activity	Strenuous physical occup.	Sedentary occup.	Moderate activity	Strenuous physical occup.	
Males										
<40	—	1	1	—	—	—	1	1	—	—
40—44	5	2	—	1	—	—	1	2	—	—
45—49	3	7	—	1	1	—	—	1	—	4
50—54	6	7	3	3	3	—	2	—	—	4
55—59	5	5	1	2	1	—	—	1	1	3
60—64	5	3	1	1	3	—	1	1	—	8
65—69	3	5	1	—	—	—	—	—	—	13
70—74	—	1	—	1	—	—	—	—	—	15
75—79	—	—	—	—	—	—	—	—	—	6
	27	51	7	9	8	—	5	6	1	53
	65			17			12			
Female										
45—49	—	—	—	1	—	—	—	—	—	1
50—54	—	2	—	—	—	—	—	—	—	1
55—59	—	—	—	—	—	—		1	—	7
60—64	—	—	—	—	—	—	—	—	—	7
65—69	—	—	—	1	—	—	—	—	—	14
70—74	—	—	—	—	—	—	—	—	—	12
75—79	—	—	—	—	—		—	—	—	4
	—	2	—	2	—	—	—	1	—	46
	2			2			1			

In the decision as to the return to work, the subjective health conditions of the patients naturally play an important role It is quite remarkable that — as seen from table VI — 109 men regarded themselves as healthy and only 40 as not healthy whereas the opposite trend was observed *in women — 17 healthy* as against 34 not healthy The mean ages at interview were respectively 62 6 and 62 years for the men and for the women 67 5 and 70 years of age.

No tendency was observed for "single" people to regard themselves as less healthy than married people. Altogether 15 men and 3 women were working although they did not consider themselves as healthy

This finding of a particularly impaired state of health in women is also substantiated by the judgement of their capacity *for work at home (table VII)* In the opinion of the interviewer the men were more apt to judge their capacity and state

Table VIII Return to work — comparison between Stockholm — Göteborg — Malmö

	No. of patients			Age groups (mean age)	Return to same work	Return to less demanding work	Not working after infarction — working before
	Males	Females	Total				
					(% of patients working before infarction)		
Stockholm	149	51	200	40—89 (64)	67 (56%)	32 (27%)	21 (17%)
					(83%)		
Göteborg	129	26	155	Up to 55 (48)	82 (55%)	49 (32%)	19 (13%)
					(87%)		
Malmö	54	31	85	40—89 (65)	29 (63%)	8 (17%)	9 (20%)
					(80%)		

In comparison with the Malmö and Göteborg-studies quoted above (3, 8) this Stockholm investigation shows a rather similar picture (table VIII).

While it may be stated that figures of a 80—85 per cent return to work in survivors of myocardial infarction, who were working before their infarct, is a very encouraging figure, it should not be forgotten that there are still many problems of social or psychological nature also for the working post-infarct patient. It is our hope that this preliminary study — the full content of which has not been analysed in this report — will assist in mapping out the types of problems, in which further medical and social aid can be usefully offered.

Summary

A preliminary report is given on the results of a follow-up study of 200 post infarct patients from the Serafimer lasarettet in Stockholm with particular emphasis on factors influencing the patients return to work.

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3. Biörck, G. & Tatlacy, E. Sociala och psykologiska problem hos patienter med hjärtinfarkt. Socialmed. T. 18: 407, 1957.

Table VII Females. Capacity in home and household

Age at interview	No activity	Same activity	Little reduced	Highly reduced
50–54	–	–	2	1
55–59	–	–	2	1
60–64	–	–	4	6
65–69	1	1	3	5
70–74	–	–	3	5
75–79	–	2	–	10
80>	–	–	2	1
	1	3	18	29

men and 8 women — wanted more rest and assistance for the women chiefly domestic service Various suggestions concerning economic matters health education and medical follow up service were given by 34 patients (31 men and 3 women) Interest in active rehabilitation procedures were more frequently expressed by men below 60 and women below 64 years of age. The request for more rest and personal assistance was more frequent in upper age groups. The answers indicate the need for a careful appraisal of every post infarct patient before attempting a rehabilitation programme

Pertinent to this discussion also are opinions expressed concerning the usefulness of a transitional period between hospital and home in a so-called convalescence home Only 26 men and 18 women have availed themselves of this opportunity with no particular preponderance for any age group Most patients seem to have declined the offer to go there and many were but too eager to return to their habitual environment

Discussion

The data presented above indicate a remarkable willingness to return to work in male infarct patients. Their employers likewise seem very helpful in facilitating this process, whereas the social institutions have been of negligible assistance. No statement can however be made on the basis of the present material as to whether return to work has a favourable effect on the patient's future health or life-expectancy A preliminary survey in our department (6) lends support to earlier observations by others, that myocardial infarctions do occur if anything, less often during working hours than during conditions of rest.

A contrasting picture is offered by the women in this series who subjectively present a more gloomy picture, with an apparent lack of zest Part of this may — at the subjective level — be due to different psychological attitudes of men and women towards a female interviewer In general this study reinforces the impression of some earlier studies (3 4) indicating the need of psychological rehabilitation particularly in female patients.

It is interesting to observe that not more than 10 per cent of the patients were positively in favour of procedures for active rehabilitation whereas almost twice as many wanted even more alleviation of existing demands on physical activity

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	No. of patients			Age groups (mean age)	Return to same work	Return to less demanding work	Not working after infarction — working before
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60–64	–	–	4	6
65–69	1	1	3	5
70–74	–	–	5	5
75–79	–	2	–	10
80>	–	–	2	1
	1	3	18	29

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the Department of Paediatrics at Kronprinsessan Lovisas Barnsjukhus
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The Pathology of the Heart in Friedreich's Ataxia

Changes in Coronary Arteries and Myocardium

By

Björn Ivemark and Claes Thorén

The association of Friedreich's ataxia and heart disease has been known since 1863 when Friedreich first described this degenerative disease. In his paper he stated that some of his original cases had marked left ventricular hypertrophy and fatty degeneration of the myocardium. Today we know that this cardiomyopathy is evidenced clinically in the electrocardiogram, and histologically as diffuse myocardial fibrosis and hypertrophy. The incidence of cardiac involvement in Friedreich's ataxia is much higher than was earlier believed. For instance, in a Swedish series, the incidence was found to be about 90 % (30).

The pathogenesis of the myocardial damage is obscure. No relationship to the disturbances of the central nervous system has been demonstrated. Nadas et al. (19) suggested, on the basis of histological findings in a 16-year-old boy that coronary artery disease might be the aetiological factor. Few histopathological studies of the heart have been reported, and have generally been confined to brief accounts in connexion with cases investigated from the neurological and neuropathological aspects.

The object of the present paper is to describe the morphological changes in the heart in four fatal cases of Friedreich's ataxia, and to attempt to correlate them to the ECG changes and clinical manifestations of the heart disease.

Case series

The material comprises 4 patients with the typical syndrome of Friedreich's ataxia. They belong to the series of 56 cases reported elsewhere (30). For practical reasons, the same case numbers have been used as in the basic series, which contains 7 other deaths in which no autopsy was performed. In the total 11 deaths in the basic series, the mean age was 7.7 years at the onset, and 28.3 years at death.

Clinical features

Some clinical data, including the cardiac symptoms, are summarized in table I and are based on various hospital records, as well as on our own observations.

Submitted for publication August 15, 1963

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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The Pathology of the Heart in Friedreich's Ataxia

Changes in Coronary Arteries and Myocardium

By

Björn Ivemark and Claes Thorén

The association of Friedreich's ataxia and heart disease has been known since 1863 when Friedreich first described this degenerative disease. In his paper he stated that some of his original cases had marked left ventricular hypertrophy and fatty degeneration of the myocardium. Today we know that this cardiomyopathy is evidenced clinically in the electrocardiogram, and histologically as diffuse myocardial fibrosis and hypertrophy. The incidence of cardiac involvement in Friedreich's ataxia is much higher than was earlier believed. For instance, in a Swedish series, the incidence was found to be about 90 % (30).

The pathogenesis of the myocardial damage is obscure. No relationship to the disturbances of the central nervous system has been demonstrated. Nadas et al. (19) suggested, on the basis of histological findings in a 16-year-old boy that coronary artery disease might be the aetiological factor. Few histopathological studies of the heart have been reported, and have generally been confined to brief accounts in connexion with cases investigated from the neurological and neuropathological aspects.

The object of the present paper is to describe the morphological changes in the heart in four fatal cases of Friedreich's ataxia, and to attempt to correlate them to the ECG changes and clinical manifestations of the heart disease.

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Case series

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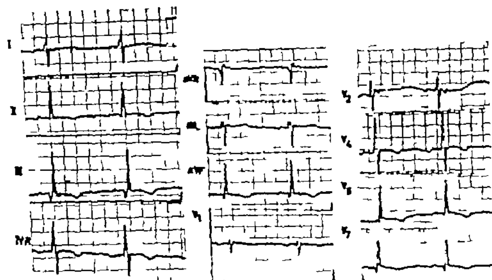


Fig. 1. Case 5 a. ECG one week before death (79 years of age). Inversion of T waves in leads II, III, VF and left precordial leads, and elevation of some S—T segments.

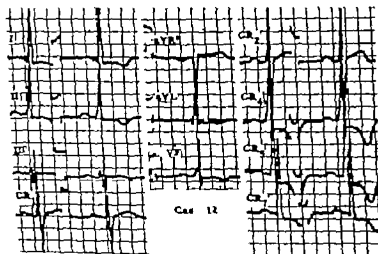


Fig. 2. Case 12. ECG two years before death (31 years of age). High voltage, deeply inverted T waves and depressed S—T segments in leads I, II, VL, VF and V_{4-7}.

Two patients who had periodic atrial fibrillation had signs of arterial embolism. Two died of pulmonary thromboembolism, and one during an attack of paroxysmal tachycardia.

ELECTROCARDIOGRAPHIC FINDINGS

The ECGs differed somewhat in all four cases. The data are summarized in table II. No patient was taking digitalis when the ECGs were recorded. The ECG changes did not

Table I. Some clinical data in 4 autopsied cases of Friedreich's ataxia

Case no	Sex	Age (yrs) at: Onset of ataxia	Loss of walking ability	Death	Body weight kg	Heart vol. ml/m B.S.A.	Cardiac symptoms: Tachy-cardia	Dysp-noea	Oede-ma	Precor-dial pain
5a	♀	7	16	29	50	—	+	—	—	—
12	♀	7	20	33	52	660	+	+	+	—
16	♂	4–5	17	21	65	440	+	+	+	+
22	♀	7	25	50	50	480	+	+	—	—
	Mean	6	19.5	33.5	54.3	526.6				

Table II. Electrocardiographic changes in 4 fatal cases of Friedreich's ataxia

Case no.	Heart rate beats/min	QRS mean axis	Q wave	S-T de-pression	T wave inver-sion	Left ventr hyper-trophy	Arrythmia: Ventr extra-systoles	Parox. supra-ventr tachyc.	Other
5a	100	+ 95	(+)	—	+	+	+	+	—
12	125	+ 35	(+)	+	+	+	+	—	2:1 block
16	75	+180°	(+)	—	—	—	+	—	Flutter Fibrillation
22	94	+175	—	—	—	—	+	+	Flutter

All four patients exhibited neurological signs typical of Friedreich's ataxia. Two of them (cases 5 a and 22) had sibs with the same syndrome, including cardiomyopathy. In case 22 progression of the neurological disease was slower than usual: thus, walking ability was lost at 25 years of age, and death occurred at 50. The two older patients (12 and 22) had diabetes mellitus of juvenile type since the age of 32 and 41 years, respectively. They had no hypercholesterolaemia, and no sign of renal or retinal involvement appeared.

In childhood, when the neurological disease was diagnosed, all four patients had a harsh systolic murmur over the pulmonary region, which at times was considered compatible with congenital heart disease. The ECG was not, however, recorded until a few years before death, when signs of cardiac disturbances appeared. The most conspicuous feature was cardiac arrhythmia with recurrent paroxysmal tachycardia, in two cases with atrial flutter and in one with atrial fibrillation. All had multifocal extrasystoles, chiefly originating in the left ventricle.

In three cases, X-ray examination of the heart was made at the same time as the ECG recording. It showed moderate enlargement, the heart volume ranging from 440–660 ml/m B.S.A. (table I).

Three patients complained of dyspnoea, and in two (cases 12 and 16) oedema developed during the last year of life. Two years before death, the last-mentioned patient suddenly experienced precordial pain radiating to the left arm, which led to admission to hospital for suspected myocardial infarction. There were, however, no laboratory findings supporting this diagnosis, and the pathological ECG remained unchanged. No other patient had any attack of precordial pain.

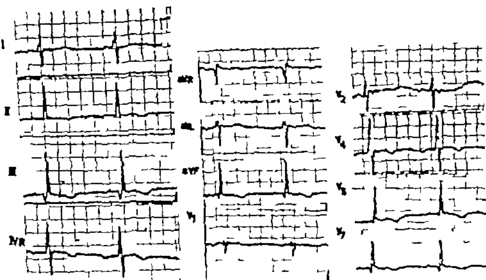


Fig. 1. Case 5 a. ECG one week before death (29 years of age). Inversion of T waves in leads II, III, VF and left precordial leads, and elevation of some S—T segments.

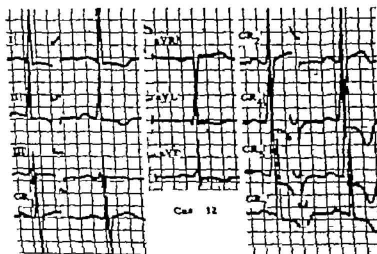


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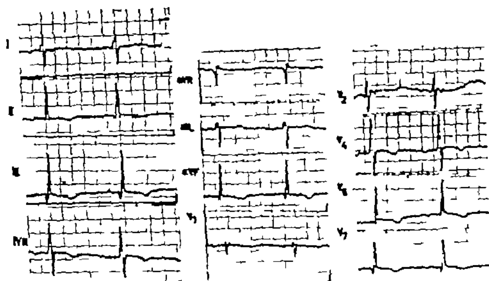


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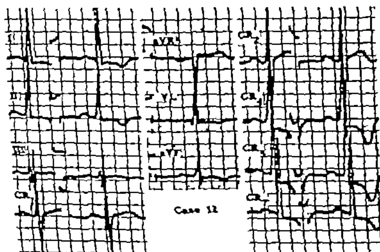


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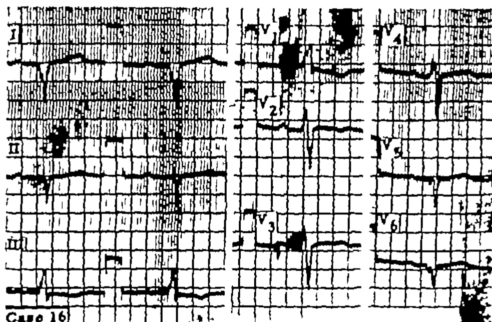


Fig 3 Case 16. ECG two years before death (19 years of age) Right axis deviation and rotation and incomplete bundle-branch block.

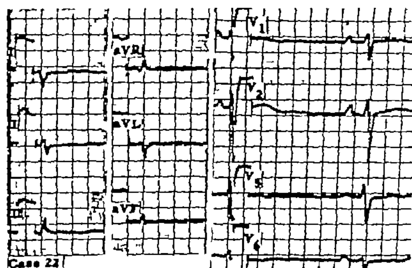


Fig 4 Case 22. ECG one year before death (49 years of age) Right axis deviation and rotation, low voltage of QRS and isoelectric T waves in all extremity leads, marked P wave in V and isoelectric T waves in left precordial leads.

alter during the last years of life, except for arrhythmia, present in every case. In no case did digitalis seem to influence the ECG changes.

All four had a normal P R interval as well as ventricular activation, QRS duration and Q-T time. Two (cases 5 a and 12) exhibited changes such as those seen in left ventricular hypertrophy with myocardial strain and damage (figs. 1 and 2) The other two (cases 16 and 22) who both had pronounced scoliosis with right convexity of the spine, showed a different picture, dominated by marked right axis deviation and unusual rotation. In case 16 there were signs of right ventricular hypertrophy (fig 3) whereas in case 22 the heart was displaced or rotated to such an extent that ordinary ECG leads did not clearly indicate the pattern of myocardial damage but hypertrophy of the right atrium (fig 4) In addition, two patients had attacks of paroxysmal supraventricular tachycardia, in one with atrial fibrillation Another had periods of alternating atrial flutter and fibrillation, and one had a 2 1 block. Ventricular extrasystoles were recorded in every case.

Table III. Structural features of the heart in 4 fatal cases of Friedreich's ataxia

Case no.	Heart				Cause of death	Myocardium		Coronary artery fibrosis	Endocard. fibro-elastosis
	Weight g	RVM mm	LVM mm	Mural thrombi		Fibrosis	Fat		
5a	?	?	?	0	Pulm. embolism	Reticular	?	++	?
12	470	5	15	0	Parox. tachycardia	Reticular	+	++	LA
16	475	8	15	LV, LA	Pulm. embolism	Reticular	+	+++	LA, RA
22	400	9	13	LA	Cerebral embolism	Reticular and patchy	+	+	LA

RVM, LVM = right and left ventricular wall thickness, the trabeculae carneae not included. ? denotes either that the feature was not recorded in the autopsy report, or that it could not be studied in the paraffin-embedded material.

Morphological changes

Since neuropathological examination disclosed changes characteristic of Friedreich ataxia in every case, no detailed account will be given of the morphological lesions of the central nervous system. Moreover the present study concerned essentially the pathology of the heart. The salient structural changes are recorded in table III.

Gross findings

Specimens were available for gross examination in three cases. All of them showed marked eccentric hypertrophy of both ventricles, the right ventricular wall measuring 5–9 mm (normal 2–3 mm) and the left 13–15 mm (normal 8–10 mm). Moderate dilatation of the atria was observed in every case, and the left atrial endocardium was yellowish-white and thickened. In cases 16 and 22, mural thrombi were present in the left atrial appendage, and in the former case similar thrombi were found in the apex of the left ventricle. The cut surface of the myocardium was moderately pale; no circumscribed areas of fibrosis or gross evidence of infarction were discernible. The larger branches of the coronary arteries, which were accessible for examination, had normal appearance, and no atheroma or thrombi were present. The pericardium was normal, and there was no evidence of congenital heart anomalies. The foramen ovale was closed in every case.

Microscopical findings

Several specimens were taken from the walls of all chambers, the septum, the area of the interventricular groove and from the coronary arteries. Specimens were also taken from the sino-atrial node and the atrioventricular bundle. The ganglion of Wrisberg could not be identified in any specimen.

The tissue was fixed in 10 % neutral formalin, and the following stains were used: haematoxylin and eosin, Verhoeff elastin, Sudan Black B, Sudan III, Pal-Weigert stain for myelin, and PAS. Fat staining was performed on fixed frozen tissue. Sudan Black B staining was done on paraffin-embedded material in one case, the only specimens available being from the right and left ventricular walls.

Pericardium and epicardial fat. No histological alterations were observed in this tissue. No inflammation was present and, with the stains used, the autonomic nerves, ganglia and fat had normal appearance.

Cardiac vessels. The veins were normal. The coronary arteries showed subintimal fibrosis in all specimens, predominantly in the large and medium-sized branches of the artery on both sides (figs. 5 and 7). No atheromatous changes were seen. The elastic membranes were usually intact, the only exception being the right main coronary artery in the oldest patient (case 22), in which the internal elastic membrane was deficient. The subintimal fibrosis of the medium-sized branches was not diffuse, but somewhat patchy. Adjacent to





Fig 5 Case 12 Cross-section of left main coronary artery The intima is greatly thickened by fibrous tissue occasionally equalling the media in thickness. Verhoeff 64 ×



Fig 6 Case 16 Medium-sized branches of the right coronary artery supplying the right atrial wall The larger branch displays marked intimal thickening whereas the small branch is normal Verhoeff. 20 ×

arteries with this feature normal arteries could be seen (fig 6) Occasionally the fibrosis was marked, narrowing the lumen to a minimal channel (fig 8) In no instance were any coronary thrombi seen, nor was there any evidence of inflammation in the vascular walls or their vicinity

Myocardium. In all specimens there was diffuse, marked, finely reticular fibrosis encircling muscle fibres which lay singly or in groups (fig 8) Occasionally a more patchy and hyaline type of fibrosis was observed, particularly in the atrial walls. The fibrosis was present in all walls — atrial and ventricular as well as septal. The myocardial fibres were hypertrophied, with large, hyperchromatic nuclei. They also displayed degenerative phenomena in the form of decreased cross-striation and slight to moderate fatty metamorphosis. The stainable fat was always localized to the myocardial fibres, and no interstitial fat was present outside the epicardium. After paraffin embedding no fat was demonstrable in sections stained with Sudan Black B. No PAS-positive material was present in the myocardial fibres. In addition, moderate interstitial oedema was present, but no inflammatory cells were observed, nor any mineral deposits There were no signs of recent myocardial infarction.

The sino-atrial node and the atrioventricular bundle had a normal appearance.

Endocardium. Subendocardial fibroelastosis of the left atrial wall was present in three cases (fig 9) The fibrous tissue component seemed to predominate, and no calcification or inflammation was visible. This lesion had the appearance of a non-specific reaction, similar to that seen in cases of long-standing elevated pressure in the left atrium. Mural thrombi of fairly recent type were present in cases 16 and 22

Microscopical changes in other organs

As stated previously all the cases displayed neuropathological changes characteristic of Friedreich's ataxia, which will not be described in detail. Various organs were studied, in addition to the heart. No conclusive evidence was obtained of any generalized muscular or vascular process.

Skeletal and smooth muscle peripheral nerves and vessels Although no systematic examination of these structures was made in the present series, in most cases tissue was preserved for a study of alterations outside the heart and central nervous system. In no case were any changes found in skeletal muscle from the psoas, intercostal spaces or diaphragm. There was no evidence of muscular dystrophy or myositis. The nerves did not appear remarkable. No vascular abnormalities were observed outside the heart. Beyond the infarcted areas (see the following) the renal vessels were

normal. This also applied to the smooth muscle of the kidneys.

Kidneys. In two cases, multiple renal infarctions of varying age were present. They seemed to originate from emboli in the heart. The occluded branches of the renal arteries displayed thrombi in various stages of organization and recanalization. No other changes were observed in the renal arteries.

Miscellaneous organs. In the two patients with diabetes, the *pancreatic tissue* showed autolysis and no definite intravital changes. In two cases with pulmonary emboli and one with heart failure, the *liver* was the site of massive congestion.



Fig. 7. Case 16. Large branch of the right coronary artery supplying the right ventricle. The thickened intima encroaches on the lumen, reducing it to minimum. Verhoeff. 64 ×

Discussion

ECG changes

The ECGs in the present patients varied in appearance. In two of them, the features were compatible with left ventricular hypertrophy and were dominated by inversion of the T waves in leads I, II, aVF and V_{4-6}, which is actually the most usual picture in Friedreich's ataxia (5, 50). The other two patients, on the contrary, showed less conspicuous ECG changes, which were dominated by right axis deviation and abnormal rotation of the heart. This can certainly be explained by the considerable kyphoscoliosis. Only vector cardiography or precordial leads from the greater part of the thorax would be able to depict the true conditions in such an extremely rotated heart as in these cases.

The difference between the ECG changes in these cases is remarkable in view of the similarity between their patho-anatomical picture. In the two patients without any appreciable thoracic deformity, the ECG was dominated by the left ventricular hypertrophy and myocardial damage, whereas the morphologically relatively greater right ventricular hypertrophy could not be demon-

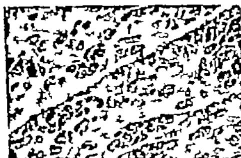


Fig. 8. Case 16. Right ventricular wall with marked reticular fibrosis separating the fibres, which lie *singly or in small groups.* Enlarged, hyperchromatic nuclei are also visible, denoting myocardial hypertrophy. Haematoxylin and eosin. 64 ×



Fig. 9. Case 12. Left atrium, showing fibroelastic thickening of the endocardium. Verhoeff. 64 ×

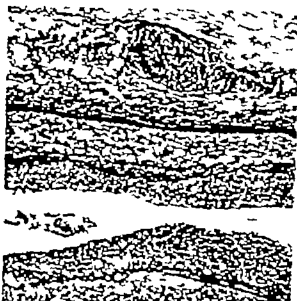


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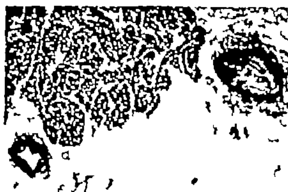


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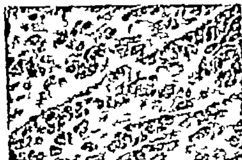


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Endocardium. Subendocardial fibroelastosis of the left atrial wall was present in three cases (fig 9) The fibrous tissue component seemed to predominate, and no calcification or inflammation was visible. This lesion had the appearance of a non-specific reaction, similar to that seen in cases of long-standing elevated pressure in the left atrium. Mural thrombi of fairly recent type were present in cases 16 and 22.

Microscopical changes in other organs

As stated previously all the cases displayed neuropathological changes characteristic of Friedreich's ataxia, which will not be described in detail. Various organs were studied, in addition to the heart. No conclusive evidence was obtained of any generalized muscular or vascular process.

Skeletal and smooth muscle peripheral nerves and vessels Although no systematic examination of these structures was made in the present series, in most cases tissue was preserved for a study of alterations outside the heart and central nervous system. In no case were any changes found in skeletal muscle from the psoas, intercostal spaces or diaphragm. There was no evidence of muscular dystrophy or myositis. The nerves did not appear remarkable. No vascular abnormalities were observed outside the heart. Beyond the infarcted areas (see the following) the renal vessels were

seems to be the most probable explanation, despite the absence of cellular infiltration (16, 25 26) We have not, however been able to find any evidence in favour of this supposition. It seems appropriate to discuss three other theories, i. e., myocardial degeneration caused by 1 A primary manifestation of a genetic disease 2 Neurological dysfunction 3. Coronary artery disease.

1 When evaluating myocardial degeneration as a primary manifestation of a genetic disease, a comparison should be made between the conditions in muscular dystrophies and familial cardiomyopathy In the latter there are no interstitial fat deposits and no changes in other types of muscles, in contrast to the findings in progressive muscular dystrophy (31)

Although isolated myocardial degeneration in families with Friedreich ataxia is conceivable (6) it has not been possible to demonstrate it with certainty despite systematic investigation (30) Familial cardiomyopathy occurs in different forms, and has been classified into three groups, mainly on morphological grounds (1) Hypertrophy with large areas of fibrosis bears the greatest resemblance to the changes in question here, although no deposits of polysaccharides have been demonstrated as described (1 2) Furthermore, a combination of two separate genetic defects is less probable than that one such defect results in several lesions.

2 The next alternative is myocardial degeneration secondary to a neurological dysfunction. Degenerative changes in bulbar nuclei, including the vagus, were demonstrated many years ago and were regarded to cause the tachycardia and cardiac arrhythmia common in Friedreich ataxia (20, 21) The heart disease was explained to be of a functional nature, caused by autonomic imbalance with overactivity of the sympathetic nervous system (11) Russell (25) was, on the other hand, unable to find any bulbar lesions in her four cases. In heredopathia atactica polyneuritiformis, a form of myocardial degeneration occurs which in all essentials, resembles that in Friedreich's ataxia, and which is presumed to be neurogenic (24) This supposition seems to be borne out by the presence of polyneuritiform lesions in e.g the vagus nerve (9)

3 In view of the ECG changes, myocardial degeneration secondary to coronary disease has often been suspected (e. g. 17) ECG changes with a rapid onset and of reversible nature have also been described (13) as well as precordial pain of angina pectoris type (12, 19 22 27) as in one of our cases. Nadas et al (19) suggested that coronary artery disease might be responsible for the myocardial damage in Friedreich's ataxia. The narrowing of the coronary arteries is probably an extremely slow process, which would explain the absence of any clinical myocardial infarction. In experimental studies on e.g the cat heart, chronic anoxia has been found to produce the same type of myocardial damage (10) The fact that these intimal changes have earlier been overlooked to such a great extent is, presumably to be ascribed to deficient histological examination Thus, in our four cases, the damage could *not* be demonstrated at gross examination, when the heart was opened routinely

The significance of these arterial changes remains poorly understood. They are supposed to be non-specific as a vascular eaction to atrophy and involution in muscle disease (12) Subintimal hypertrophy in coronary arteries is, however not described in progressive muscular dystrophy with myocardial involvement,

strated on the ECG Nor could the fibroelastosis of the atria be traced in any P wave changes more than hypertrophy in one case although it was the probable cause of the atrial arrythmia of various types present in all four cases. With such extensive myocardial damage as in these cases, one could also have expected a prolonged ventricular activation time and a lengthened Q—T interval but these changes were lacking The diffuse, generalized spreading of the myocardial fibrosis might perhaps explain a normal polarization rate.

Pathology and pathogenesis

Fibroelastosis of the atrial endocardium may have been secondary to the myocardial fibrosis, combined with raised pressure in the atria. Pressure measurements were not however made in these patients, but in the other cases in the series raised pressure has been demonstrated in both atria as well as elevated filling pressure in the right ventricle, due to increased resistance to filling explainable by rigidity of the ventricular wall (30)

Mural thrombosis is thought to be caused by fibroelastosis (4) It is, however doubtful whether fibroelastosis was of any pathogenetic importance for the parietal thrombi present in the atria in our cases. It seems likely that arrhythmia with atrial fibrillation and flutter played a greater role. The thrombus formation in the left ventricle demonstrated in one case was, on the other hand presumably of endocardial origin Arterial embolism with for example, renal and splenic infarctions can presumably explain some of the attacks of abdominal pain and fever during the last months of life that have not infrequently been described (5 18) as well as endarteritis (18, 23)

The structural changes in the heart, which were common to all the present cases, consisted of diffuse myocardial fibrosis and a varying degree of subintimal fibrosis of the coronary arteries. The literature contains only a few cases in which a histological study has been made of the coronary arteries. Intimal thickening was reported in two cases (19 28) In a third case, gross examination disclosed thrombosis of the left main coronary artery but the histological features were not described (5) Russell (25) reported the gross finding of considerable diffuse atheroma sometimes constricting but nowhere occluding main coronary arteries and their branches" in a 21 year-old man but she did not give an account of microscopical findings nor of the appearance of the coronary vessels in her other three cases.

Coronary artery changes were stated to be ruled out in one case, on the basis of post mortem coronary angiography (14) However since the authors did not mention the pressure of injection nor any histological examination of the vessel walls this observation is not relevant. It would be possible to study the structure of the vessel walls and their course by means of stereomicroangiography and serial sectioning (15)

In view of the not uncommon occurrence of diabetes mellitus in Friedreich's ataxia (29) as in two of our patients, a diabetogenic angiopathy is conceivable. However the intimal changes had a different appearance, and there was no evidence of vascular lesions in the kidneys or fundi.

Many theories have been put forward regarding the relationship between Friedreich's ataxia and heart disease. As far as the nature of the myocardial changes is concerned, an infectious or toxic cause

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and has not been observed in *myocardial* fibrosis of other aetiology

An intimate relationship does, however exist between the neurological and the cardiac degenerative processes. There may be a combination of neurological dysfunction and coronary artery or myocardial disease. Consequently careful histological studies of the vegetative nervous centres and experimental studies will perhaps explain the pathogenesis of the cardiopathy in Friedreich's ataxia

Summary

An account is given of four fatal cases of Friedreich's ataxia with cardiomyopathy Initially they were all believed to have congenital heart disease, but the systolic murmur subsequently disappeared and during their last years of life arrhythmia was the dominant cardiac symptom. Dyspnoea and precordial pain occurred Cerebral or pulmonary embolism was the cause of death in three cases

In two patients the electrocardiographic changes were indicative of left ventricular hypertrophy with myocardial damage The other two who had marked thoracic deformity showed pronounced right axis deviation and rotation without signs of myocardial damage

A correlative study of the autopsy findings and ECG data shows poor agreement. Thus, in every case, the heart shows generalized enlargement with hypertrophy of both ventricles, most prominent in the right. There is diffuse, reticular myocardial fibrosis of the walls of all chambers, and signs of non-inflammatory degeneration of myofibrils.

In every case the main and medium-sized branches of the coronary arteries display subintimal fibrosis with a varying degree of narrowing of the vessel lumen. The consistent finding of narrowed coronary arteries is supposed to contribute to the myocardial damage in Friedreich's ataxia. The pathogenesis of the cardiopathy is discussed but remains obscure.

Acknowledgement

The present investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases.

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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The Electrocardiogram and the Frontal Vectorcardiogram in Ostium Secundum Defect and Endocardial Cushion Defect

By

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Atrial septal defects fall into two main groups, the septum secundum defects and the septum primum defects. The first group stems from faults in the development of the fetal interatrial partitioning named the septum secundum, while the second group is related to abnormal development of another interatrial embryological structure, the septum primum. The septum secundum defects, which comprise approximately 65 per cent of the abnormal interatrial communications, are usually located in the region of the fossa ovalis.

The septum primum defects are located anterior to the fossa ovalis. Since the primum defects reach the periphery of the atrial septum in the region between the atrioventricular valves, a muscular brim is partly lacking and concomitant malformations of the atrioventricular valves are common. In some cases the abnormal septal opening involves also the uppermost part of the interventricular septum. When the defect in the interventricular septum is marked and there is a deep cleft in the atrioventricular leaflet on each side of the defect, the malformation is manifest as a common atrioventricular canal. Both clinically and anatomically there is a gradual transition from the simpler forms of septum primum defects to the more severe abnormality of a *common* atrioventricular canal. These defects are therefore often grouped together under the term endocardial cushion defects. The endocardial cushions are tissue proliferations which appear during the 6th week of embryonic life on the ventral and dorsal part of the atrioventricular canal. Later these cushions fuse in the midline and form the aortic leaflet of the mitral valve, the septal leaflet of the tricuspid valve, the septum membranaceum and a small part of the interatrial septum close above the atrioventricular valves.

Campbell and Missen (7) grade the endocardial cushion defects as follows.

Submitted for publication August 23, 1963.

Table I. Twelve cases of pure secundum defect

Case no.	Age	Sex	Verified by	Â QRS degrees	P R (sec)	V -pattern
1	1 month	♀	Necroscopy	+ 150	0.12	—
2	4 months	♀	Necroscopy	+ 140	0.08	—
3	9 years	♀	Op.	+ 135	0.17	rsR
4	12 years	♂	Op.	— 40	0.16	RSr
5	15 years	o	Op.	+ 140	0.14	R
6	18 years	♀	Op.	+ 125	0.20	qR
7	20 years	♀	Op.	+ 120	0.14	rSR
8	30 years	♀	Op.	+ 150	0.16	qR
9	38 years	o	Op.	+ 80	0.18	rSR
10	41 years	♀	Necroscopy	+ 90	0.12	rS
11	42 years	♀	Op.	+ 100	0.16	qR
12	70 years	♂	Necroscopy	+ 120	Atrial fibrillation	rsR

Table II. Nine cases of secundum defect with concomitant cardiac lesions verified by necroscopy

Case no.	Age (days)	Sex	Concomitant lesion	Â QRS	P R	V pattern
1	2	♂	Transposition of the great vessels	+ 140	0.12	Rs
2	17	♂	Transposition of the great vessels	+ 120	0.10	—
3	40	♂	Transposition of the great vessels	+ 115	0.10	—
4	40	♀	Transposition of the great vessels	+ 120	0.08	Rs
5	40	♀	Transposition of the great vessels	+ 125	0.12	—
6	60	o	Transposition of the great vessels	+ 120	0.11	Rs
7	14	♀	Pulmonary stenosis	+ 120	0.10	qRs
8	5	♂	Aortic atresia	+ 120	0.10	—
9	16	♂	Aortic atresia	+ 130	0.10	—

Material and methods

The electrocardiograms of 34 patients with atrial septal defects have been examined. In all cases the diagnosis has been proved surgically or by necropsy. Endocardial cushion defects were found in 13 cases, in three of which concomitant cardiac lesions were present. Bicuspid aortic valve, ductus arteriosus and pulmonary stenosis were found in cases no. 7, 11 and 13 respectively. In the remaining 21 cases an ostium secundum defect was present. In 9 cases of ostium secundum defect other cardiac malformations were also met with. Further particulars are shown in tables I—III.

Frontal plane vectorcardiograms were constructed from the standard leads and/or unipolar limb leads. Care was taken to avoid errors caused by differences in timing between the different leads. The smallest fault in timing may produce large aberrations in the constructed loops. In each case the vector loop has been constructed three times with a minimum interval of one week. Only trifling differences could be detected on comparing the three versions of each loop.

The mean electrocardiographic axis has been obtained from the vectorcardiogram and from algebraic determinations of the areas covered by the QRS-complexes in lead I and III.

Endocardial cushion defect grade I Persistent ostium primum	Ostium primum. Bifid anterior mitral valve-cusp.
Endocardial cushion defect grade II	Ostium primum. Clefts in both the mitral, anterior and tricuspid septal valve-cusp
Endocardial cushion defect grade III Persistent common A V canal	Common A V canal

Wakai and Edwards (18) prefer the name persistent common atrioventricular canal for the whole group and distinguish between partional transitional and complete forms. Poul (14) groups the first two of these forms together under the term persistent ostium primum and calls the third one persistent common atrioventricular canal

Previously the differentiation of ostium secundum defects from endocardial cushion defects was of academic interest only Through the advances of cardiac surgery the preoperative distinction between these two types of malformation has achieved great practical importance during the last few years The ostium secundum defects may be closed without the use of a heart lung machine while in the correction of endocardial cushion defects some form of cardiopulmonary by pass is necessary In the differential diagnosis between the two main forms of atrial septal defect electrocardiography has been shown to be a most important tool.

In 1956 Blount et al (4) described left axis deviation in 3 of 4 cases of endocardial cushion defect Toscano-Barbosa et al (16) described the electrocardiographic and vectorcardiographic pattern in 16 cases of this type, all proved by necropsy or operation. The mean electrical axis, Â QRS had a direction between — 90 and — 115 in 9 cases between — 135 and — 175 in 5 cases In 2 cases the direction of the Â QRS was — 60 The vectorcardiographic pattern was characteristic and had a basic uniformity In the frontal plane the QRS vector advanced in a counterclockwise direction and was usually mainly superior to the isoelectric point.

In 97 of 99 cases of ostium secundum defects proved by autopsy or operation Toscano-Barbosa et al. (17) found the Â QRS in the range + 80° to — 160 In one patient the direction of Â QRS was — 30 and in another it was — 60 The QRS loop in the frontal plane showed a clockwise direction in 92 cases and was located mainly inferior to the isoelectric point In one patient the loop was counterclockwise directed and situated above the isoelectric point whilst in 6 cases it ran a figure-of-eight course approximately parallelling the isoelectric line. In 5 of the 7 patients with vector cardiograms which did not conform to the usual pattern the defect was located just above the atrioventricular valves in the lowest part of the septum.

The findings of Toscano-Barbosa have been confirmed by a few other authors (2 3 5 9 15) In several of these publications however there has been inadequate checking of the diagnosis by autopsy or operation even when all cases are registered as "proved It is not always clear which cases are controlled and which are not.

In this paper a report is given on electrocardiographic and vectorcardiographic aspects in 34 cases of atrial septal defect.



Fig 1. Frontal vectorcardiograms in 12 cases of isolated ostium secundum defect.

present in all of them. In two of these cases the electrocardiograms were compatible with left ventricular hypertrophy At necropsy left ventricular hypertrophy was evident in both. In one of the cases the left ventricular hypertrophy was probably the result of gross mitral incompetence caused by a cleft in the aortic leaflet of the mitral valve. The second case was a grade III endocardial cushion defect.

The A QRS in the 13 cases of endocardial cushion defects, was located as shown in table III In all but three of these cases the direction of the Â QRS was between — 30° and — 140° In the remaining cases, no. 3 7 and 9 it was + 140° + 50° and + 30° respectively In 10 of the cases of endocardial cushion defects the frontal plane vector loop advanced in a counterclockwise direction with the main part of the loop usually superior to the isoelectric point. In the remaining 3 cases the frontal plane vectorcardiogram had a figure-of-eight configuration with approximately the same area above and below the isoelectric point. One of our cases no. 5 had both ostium primum and ostium secundum defect. The vector loop progressed in a counterclockwise manner (fig 3)

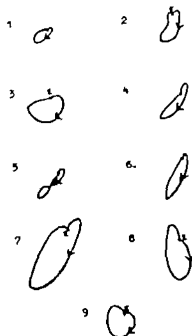


Fig 2. Frontal vectorcardiograms in 9 cases of ostium secundum defect with concomitant cardiac lesions.

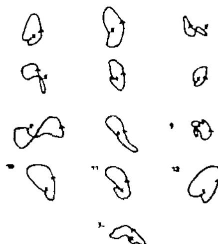


Fig 3. Frontal vectorcardiograms in 13 cases of endocardial cushion defect.

Table III Thirteen cases of endocardial cushion defect

Case no.	Age	Sex	Verified by	Grade of lesion	Â QRS	P R (sec)	V pattern	R_1 (mm)
1	1 month	♂	Necroscopy	I	− 75	0.12	—	
2	6 months	♂	Necroscopy	I	− 40	0.15	rR	17
3	6 months	♂	Necroscopy	I	+140	0.15	R	10
4	4 years	♀	Op.	I	−140	0.17	rSR	16
5	10 years	♂	Op.	I	− 30	0.18	rsR	17
6	35 years	♀	Op	I	− 60	0.20	rsr	11
7	11 months	♂	Necroscopy	II	+ 50	0.14	rSR	29
8	6 years	♀	Op.	II	− 30	0.20	rsR	35
9	8 years	♂	Op.	II	+ 30	Nodal rhythm	—	—
10	1 month	♂	Necroscopy	III	− 85	0.14	qRs	10
11	4 months	♀	Necroscopy	III	− 45	0.11	Rs	5
12	4 months	♂	Necroscopy	III	− 70	0.14	qR	15
13	8 months	♂	Necroscopy	III	− 85	0.12	qR	7

Results

In the 12 cases of pure secundum defect the P R interval was prolonged in three, and in one case it was in the upper normal range, when compared with the values given by Ziegler (20) In 9 cases of pure secundum defects precordial leads were available. In 5 of these the V revealed incomplete right bundle branch block and a qR pattern was present in 3 In only one was the QRS complex of the V_1 lead normal

In 11 of the cases of isolated ostium secundum defect the direction of the Â QRS was between + 80 and + 140 and the frontal plane vectorcardiograms progressed in a clockwise direction (fig 1) The main part of the loop was inferior to the isoelectric point In one case however the direction of the Â QRS was — 40 and the vector loop was of the counterclockwise type with the main part of the loop above the isoelectric point. Preoperatively this patient was believed to suffer from endocardial cushion defect.

The P R interval was prolonged in only three of 9 cases of ostium secundum defects combined with other congenital cardiac lesions in which the interatrial communication was of minor importance compared to the concomitant defects. However the Â QRS was between + 115 and + 140 The frontal vector loop advanced in a clockwise direction in 8 of these patients (fig 2) In the remaining case the loop had a figure-of-eight configuration In all 9 cases the main part of the frontal vector loop was inferior to the isoelectric point.

The P R interval was widened in 7 of the 13 cases of endocardial cushion defects. In the other 6 cases the P R interval was in the upper normal range In case no 8 operation revealed a gross cleft in the aortic leaflet of the mitral valve. The lateral precordial leads in this case were compatible with left ventricular hypertrophy Unipolar precordial electrocardiograms were available in 6 autopsied cases of endocardial cushion defects. In all these cases the electrocardiograms were compatible with right ventricular hypertrophy and this was found to be



Fig 1 Frontal vectorcardiograms in 12 cases of isolated ostium secundum defect.

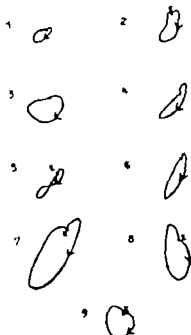


Fig 2 Frontal vectorcardiograms in 9 cases of ostium secundum defect with concomitant cardiac lesions.

present in all of them. In two of these cases the electrocardiograms were compatible with left ventricular hypertrophy At necropsy left ventricular hypertrophy was evident in both. In one of the cases the left ventricular hypertrophy was probably the result of gross mitral incompetence caused by a cleft in the aortic leaflet of the mitral valve. The second case was a grade III endocardial cushion defect.

The Â QRS in the 13 cases of endocardial cushion defects, was located as shown in table III In all but three of these cases the direction of the Â QRS was between — 30° and — 140° In the remaining cases no. 3 7 and 9 it was + 140° + 50° and + 30° respectively In 10 of the cases of endocardial cushion defects the frontal plane vector loop advanced in a counterclockwise direction with the main part of the loop usually superior to the isoelectric point. In the remaining 3 cases the frontal plane vectorcardiogram had a figure-of-eight configuration with approximately the same rea bove and below the isoelectric point. One of our cases, no. 5 had

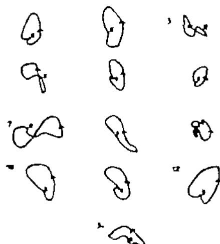


Fig 3. Frontal vectorcardiograms in 13 cases of endocardial cushion defect.

both ostium primum and ostium secundum defect. The vector loop progressed in a counterclockwise manner (fig 3)

Table III Thirteen cases of endocardial cushion defect

Case no.	Age	Sex	Verified by	Grade of lesion	Å QRS	P R (sec)	V_1 pattern	R_1 (mm)
1	1 month	♂	Necroscopy	I	− 75	0.12	–	–
2	6 months	♂	Necroscopy	I	− 40	0.15	rR	17
3	6 months	♂	Necroscopy	I	+140	0 15	R	10
4	4 years	♀	Op.	I	−140	0.17	rSR	16
5	10 years	♂	Op.	I	− 30	0.18	rsR	17
6	35 years	♀	Op	I	− 60	0.20	rsr	11
7	11 months	♂	Necroscopy	II	+ 50	0.14	rSR	29
8	6 years	♀	Op.	II	− 30	0.20	rsR	33
9	8 years	♂	Op.	II	+ 30	Nodal rhythm	–	–
10	1 month	♂	Necroscopy	III	− 85	0.14	qRs	10
11	4 months	♀	Necroscopy	III	− 45	0 11	Rs	25
12	4 months	♂	Necroscopy	III	− 70	0.14	qR	15
13	8 months	♂	Necroscopy	III	− 85	0.12	qR	7

Results

In the 12 cases of pure secundum defect the P R interval was prolonged in three, and in one case it was in the upper normal range, when compared with the values given by Ziegler (20) In 9 cases of pure secundum defects precordial leads were available In 5 of these the V_1 revealed incomplete right bundle branch block, and a qR pattern was present in 3 In only one was the QRS complex of the V_1 lead normal

In 11 of the cases of isolated ostium secundum defect the direction of the A QRS was between + 80 and + 140 and the frontal plane vectorcardiograms progressed in a clockwise direction (fig 1) The main part of the loop was inferior to the isoelectric point. In one case, however the direction of the Å QRS was — 40 and the vector loop was of the counterclockwise type with the main part of the loop above the isoelectric point Preoperatively this patient was believed to suffer from endocardial cushion defect.

The P R interval was prolonged in only three of 9 cases of ostium secundum defects combined with other congenital cardiac lesions in which the interatrial communication was of minor importance compared to the concomitant defects. However the Å QRS was between + 115 and + 140 The frontal vector loop advanced in a clockwise direction in 8 of these patients (fig 2) In the remaining case the loop had a figure-of-eight configuration. In all 9 cases the main part of the frontal vector loop was inferior to the isoelectric point.

The P R interval was widened in 7 of the 13 cases of endocardial cushion de fects. In the other 6 cases the P R interval was in the upper normal range. In case no 8 operation revealed a gross cleft in the aortic leaflet of the mitral valve. The lateral precordial leads in this case were compatible with left ventricular hyper trophy Unipolar precordial electrocar diograms were available in 6 autopsied cases of endocardial cushion defects. In all these cases the electrocardiograms were compatible with right ventricular hypertrophy and this was found to be

For the determination of the Â QRS the method commonly described in text books is based on simple arithmetic calculations from the positive and negative amplitudes of the QRS complex. This may often be sufficient for clinical purposes, but it may sometimes lead to grossly misleading results as shown in two cases in table IV When axis determinations are used in the differential diagnosis of the different types of atrial septal defects, determinations from area calculations must therefore be insisted on.

A word of caution is also necessary concerning the commonly used expressions right axis deviation and left axis deviation. Some authors (11 12) consider directions between — 30° and — 90° as left axis deviation. Others extend the expression left axis deviation to include the sector between — 30° and — 150° and will only accept values between + 90° and — 150° as right axis deviation (6) Using the former definition many cases of endocardial cushion defects will show right axis deviation electrocardiographically Toscano-Barboza 16 cases exemplify this. In 14 of these cases the direction of Â QRS is recorded as right axis deviation, and left axis deviation is recorded in only cases According to the second definition 11 of the 16 cases would be registered as showing left axis deviation and the cases of right axis deviation would be reduced to 5 Owing to variable definitions of the terms left and right axis deviation their use is apt to cause confusion, and should be discouraged The direction of the Â QRS should be given in degrees only

Variable definition of endocardial cushion defects and septum secundum defects may also cause confusion Wakai and Edwards (18) claim that t least a trace of left n the aortic cusp of the mitral valve is obligatory for the diagnosis of endocardial cushion defect. A low position of the defect in the septum is not enough. Defects situated just above the atrioventricular valves are however considered as endocardial cushion defects by other workers even when not the slightest indentation of the aortic leaflet of the mitral valve (13) can be traced. It is therefore not unlikely that some of the 5 patients of Toscano-Barboza et al. (17) with low septal defects registered as cases of septum secundum defects, and showing vectorcardiographic patterns unusual for this condition might have been registered as primum defects by other authors. Regardless of the most correct definition from an embryological point of view t may be useful for practical reasons to consider the low and anterior defects which reach the valvular ring as belonging to the same group The usual technique for closing septum secundum defects will often not suffice in these cases, and cardiopulmonary bypass must be resorted to Clinically such cases are therefore best grouped with the endocardial cushion defect cases.

Regardless of such borderline cases, however there can be no doubt that not only the axis deviation, but in rare cases also the vectorcardiographic loop considered typical for septum primum defects may indeed be seen in cases of septum secundum defects with the opening in the region of the fossa ovalis or above (17) Our case no. 4 is an exampl of the diagnostic difficulties in cases of this type. Preoperatively the patient was believed to have a septum primum defect, but the cardiac surgeon made the unqualified tatement that it was a septum secundum defect. It is striking, however that the defect was localized in the lower part of the atrial septum, and that, in the

Table IV. Â QRS calculation in 13 cases of endocardial cushion defect

Case no.	A	B	C
1	− 75	− 80	−115
2	− 40	− 35	− 60
3	+140	+130	+120
4	−140	−140	− 80
5	− 30	− 30	− 40
6	− 60	− 60	− 65
7	+ 50	+ 45	+ 40
8	− 30	− 40	− 70
9	+ 30	+ 25	− 80
10	− 85	− 85	− 60
11	− 45	− 50	− 90
12	− 70	− 70	− 85
13	− 85	−120	+175

A. Determination by algebraical calculation of the areas under the QRS complex in lead I and III.

B. Derived from vectorcardiograms.

C: Determination by algebraical calculation of the amplitudes of the QRS complex in lead I and III.

Discussion

When an atrial septal defect has been diagnosed the next question which must be answered is whether this defect is a simple septum secundum defect or some form of endocardial cushion defect. The severity of the symptoms presence of signs indicating involvement of atrio-ventricular valves hemodynamic observations during cardiac catheterization roentgenologic and angiographic observations may all be of value for the differential diagnosis, but the most precise information on this point is usually yielded by the electrocardiographic examination and especially by the vector cardiogram.

Signs of left atrial hypertrophy and dilation — a wide and notched P wave in the standard leads and a diphasic P wave with prominent negative deflection in V_1 — are seen in a minor group of cases of endocardial cushion defects, owing to the occurrence of concomitant mitral insufficiency. These electrocardiographic findings were seen in only one of our cases of endocardial cushion defects (case no. 11). They are even more rare in cases of septum secundum defect and accordingly are not without a certain value for the differential diagnosis.

In our material the tendency towards prolongation of the P R interval was more marked in the cases of endocardial cushion defect. In no case of septum secundum defect did the P R interval exceed the normal maximum values given by Ziegler (20) and in only about one-fourth of the cases were the values for the P R interval in the upper part of the normal range. Definitely prolonged P R intervals were found, however in *about one half of the endocardial cushion* defect cases, and in all the remaining cases of this type the P R interval was in the upper part of the normal range. Though described in cases of septum secundum defect (10) a definitely prolonged P R interval may also be a feature of interest in the electrocardiographic differentiation between cases of endocardial cushion defects and septum secundum defects.

The appearance of the ventricular complex is much the same in the medial precordial leads in cases of endocardial cushion defect and cases of septum secundum defect. The occurrence of signs of left ventricular hypertrophy in the lateral precordial leads in cases of atrial septal defects indicates the possibility of mitral insufficiency and accordingly is a point in favor of an endocardial cushion defect. Such findings were met with in 3 of our group of atrial septal defects of this type but in none of our secundum defect cases.

The most important electrocardiographic features relevant to the differential diagnosis of the two main forms of atrial septal defect are stressed.

In the ostium secundum defect cases the Â QRS was between + 80 and + 140 degrees in all but one, where it was — 40 degrees. In 13 cases of endocardial cushion defect the Â QRS was between — 30 and — 140 degrees in 10 and in the remaining cases it was between + 30 and + 140 degrees.

Our vectorcardiographic findings, based upon standard electrocardiograms, revealed a quite uniform frontal vector loop pattern in all but one of the secundum defect variety. The loop was mainly inferior to the isoelectric point and the direction of inscription was clockwise.

In 13 cases of endocardial cushion defect the frontal vector loop was mainly superior to the isoelectric point with a counterclockwise direction of inscription in 10 while the remaining 3 cases had a figure-of-eight configuration, with approximately the same area above and below the isoelectric point.

It is concluded that vector loop constructions are more valuable than Â QRS determinations for the differentiation between septum secundum defect and endocardial cushion defect. Three of our endocardial cushion defect cases with Â QRS between + 30 and + 140 degrees had a frontal vector loop which varied from that characteristically seen in ostium secundum defect.

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surgeon's opinion a slight degree of tricuspid incompetence was present. To the best of our knowledge, however no case of an endocardial cushion defect with the loop considered typical for ostium secundum defects is recorded though in 2 of our 13 cases the loop had an horizontal location. In these two cases a normal axis direction was met with.

It is noteworthy that the case which had a combination of ostium secundum and ostium primum defect revealed the vectorcardiographic configuration typical for endocardial cushion defects

In addition to cases of endocardial cushion defects the vector loop considered typical for this lesion may also be seen in cases of acquired heart disease. It has been described in cases of coronary sclerosis (19) hypertensive heart disease (12) combined right bundle branch block and left ventricular hypertrophy (1) and in chronic cor pulmonale (12)

This scarcely detracts from its value in the differential diagnosis in cases of atrial septal defects, but it is noteworthy that the characteristic endocardial cushion defect vector loop has also been described in other forms of congenital heart disease such as tricuspid atresia and ventricular septal defect (8) These findings are in agreement with our experience We have also found the same vector pattern in several other types of congenital heart diseases such as uncomplicated cases of severe pulmonary stenosis ductus arteriosus persistens, tetralogy of Fallot and transposition of the great vessels without atrial septal defects.

Even though this type of loop may be met with in exceptional cases of secundum defect, and may exceptionally be lacking in endocardial cushion defects, and even though it may occasionally be found in other types of congenital heart disease, it is a finding which has a comparatively high degree of specificity and is a most valuable adjunct for the important differentiation between septum secundum defects and endocardial cushion defects.

Our results indicate that vector loop constructions are of more value than determination of Â QRS for this differentiation. Three of our endocardial cushion defect cases had an Â QRS between + 30 and + 140 degrees. All of these had frontal vector loops which varied from that characteristically seen in ostium secundum defects.

In most of the papers which have appeared concerning the vectorcardiographic pattern in cases of endocardial cushion defects, the loops have been obtained by vectorcardiographs. Our findings based upon standard electrocardiograms, are in agreement with those obtained by the expensive vectorcardiographic outfits. The statement is often met with that vector loop construction from electrocardiograms is time-consuming inaccurate and impractical for clinical use. We are not in agreement with this. With some training frontal loop construction takes approximately 5 minutes and our results demonstrate that the method is at least adequate for differentiation between the two main forms of atrial septal defect.

Summary

Electrocardiograms and frontal vectorcardiograms from 12 cases of isolated ostium secundum defect 9 cases of ostium secundum defect combined with other congenital cardiac lesions and 13 cases of septum primum and other endocardial cushion defects have been examined All cases have been verified by operation or necropsy

Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

From the Medical Clinic B (Renal Clinic) Lund University Lund, Sweden

Short Clotting-time During Haemodialysis by Heparinization with an Infusion Apparatus

Preliminary Report

By

Bengt Lindqvist, Hans Fritz, Karl Erik Hagstam, Harry Lecerof and Bengt Liljenberg

As a guide for determining the dosage of heparin during haemodialysis at this clinic Piper's (1) method for measuring clotting-time has been used since 1946. A clotting-time of 30—45 minutes for arterial blood is generally considered expedient. In some cases with a severe tendency to bleed and/or heavy active bleeding, a shorter clotting-time is desirable. Since June, 1962, we have in such selected cases used continuous administration of heparin in small doses, without giving protamine-sulphate — altogether in 38 dialysis treatments, the average clotting time in the arterial blood being 7—14 minutes.

Technique

Before dialysis is begun, the cellophane tube of the machine (the Alwall model) is filled with citrated blood. The electrolyte solution does not contain any calcium. When the blood begins to flow from the artery, the heparin solution is infused (1 mg/ml/min.) into the tube that conveys the blood to the machine. Calciumchloride is added to the dialysing fluid about three-quarters of an hour after the start of dialysis.

The clotting-time is measured 10—20 times during the 6—8 hour dialysis. Samples are taken with a thin, sterile, and dry needle from the arterial blood just as it leaves the patient (sample A) and from the blood that has passed through the cellophane tube (sample B). If the clotting-time in sample A exceeds 14 min., the speed of heparin infusion is reduced, unless the clotting-time in sample B is shorter than 8 min.

The infusion apparatus was the model Unita II" Braun AG, West Germany designed for continuous infusion of heparin through two long 5 ml syringes which, by turns, are automatically filled from a bottle

Submitted for publication November 4, 1963.

Results and comments

The average clotting-time in arterial blood in sample A was, for the 38 dialysis treatments, 12 (7—14) min. In 4 cases the clotting time never exceeded 10 min. (fig. 1), in 5 it was never longer than 14 min. and in the rest never longer than 35 min. The average clotting time in

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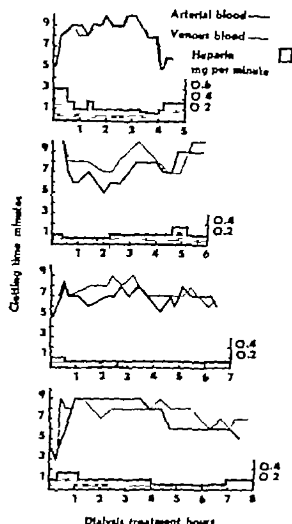


Fig 1 Heparinization. Clotting time of arterial and venous blood, respectively

sample B was 15 (8—23) min The efficacy of the artificial kidney was not reduced

With the technique described here haemodialysis can be carried out in patients with active severe bleeding or a marked tendency to bleed the clinical value of the technique in a larger series of patients will be reported later

Summary

The clotting time measured by Piper's method in arterial blood during heparinization with an infusion apparatus could be kept as short as 12 min. this being the average for 38 haemodialyses In 4 treatments the clotting time did not exceed 10 min on any occasion. No use was made of a heparin antidote. Heparinization by this technique seems to reduce the risk of bleeding and is therefore recommended in patients with active bleeding or haemorrhagic diathesis.

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Acta Medica Scandinavica. Vol. 175, fasc. 2, 1964

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The Sodium, Potassium and Water Content of Liver Tissue

By

J H Atterhög B Håkansson S A. Johansson and I G Porjé

Serum and tissue electrolyte levels in different diseases and particularly in those of the cardiovascular system have been studied intensively in recent years. Determinations of sodium and potassium levels in serum are done routinely nowadays but give no information about tissue levels of electrolytes or water in physiological or pathological states. Since the introduction of modern thiazide diuretics, the effects of various forms of treatment on electrolyte metabolism have also been studied.

Sodium seems t be important for the occurrence of oedema and for the initiation and maintenance of hypertension. Comparisons of regions with different levels of salt consumption have demonstrated an association between high salt consumption and a high incidence of arterial hypertension. Increased N and water levels in liver tissue have also been demonstrated in patients with arterial hypertension (8) Experiments with ²⁴Na indicate that hypertensives have larger reserves of exchangeable N than normotensives (5 6) High levels of Na, water and mucopolysaccharides has also been found in the vessel walls of animals with experimentally induced hypertension (1 9 13) There are, however only a few reports dealing with the electrolyte levels in various organs of human beings (5 8) No systematic studies of the amounts of water and electrolytes in human liver tissue seem to have been carried out in spite of the central position of the liver as a store of many substances. In some diseases, cardiac decompensation for example, there is enlargement of the liver A high water content has been demonstrated in liver tissue obtained from small group of patients with signs of decompensation (8) Here we are going to present the results of electrolyte determinations on liver tissue from groups of patients with decompensation nd some other diseases.

Material

Liver biopsies were performed on 29 patients from the Geriatric Clinic, Södersjukhuset, Stockholm. Patients of both sexes were included and the mean age was 68 years with the youngest 40 and the oldest 84 years old. All patients were examined by electrocardic

Submitted for publication August 22, 1963

graphy cardiac radiography and urography as well as for signs of liver or kidney disease. Most patients had normal renal function. Plasma creatinine levels were within the normal range and urographical studies were normal. Prothrombin-proconvertin activity and thrombocyte counts were determined before biopsy The patients were divided into four groups — "normal "oedema" which included patients with cardiac decompensation and palpable hepatomegaly hypertension and liver cirrhosis without hypertension or oedema. The normal group consisted of patients without hypertension or signs of cardiac, hepatic, or pulmonary disease. The hypertensive group was also examined by ophthalmoscopy

After the first biopsy 16 patients were given either chlorothiazide (chlotride®) or a placebo for 14 days. The patients were selected at random without consideration of the previous groupings and divided into two new groups 7 received chlotride and 9 the placebo. A second liver biopsy was taken after 14 days and examined in the same manner as the first

Methods

After premedication with 5 mg dihydrohydroxycodeinone, liver biopsies were taken with a Vim-Silberman needle. The liver tissue obtained was examined both microscopically and chemically The tissue was weighed on a Kahn electrobalance and the dry material determined after heating for 24 hours at 90° C. The Na and K levels were determined on ashed liver tissue using an EEL model A flame photometer The amounts of Na, K, and water were related to dry material levels of Na and K are expressed as mEq and water in g The ratios Na/K and Na + K/water have also been calculated

Results

Table I lists the mean values calculated for each group and gives the number of patients in each group The mean values for the normal group (n = 7) were 0 22 mEq Na 0.21 mEq K, and 2 11 g water The mean Na/K ratio was 1 11 and the Na + K/water ratio was 0.212

Liver tissue from the oedema group (n = 4) contained 0.37 mEq Na, 0.28 mEq K and 3 7 g water Na/K = 1.32 and Na + K/water = 0 177 Patients with cardiac decompensation then, had significantly more Na and water and possibly more K in their liver tissue than did patients in the normal group. The mean values for Na/K and Na + K/water were higher than those of the normal group (table I)

Liver tissue from patients with arterial hypertension (n = 12) had mean levels of 0.34 mEq Na 0.25 mEq K and 2.73 g water The mean values for the ratios Na/K and Na + K/water have been calculated and included in table I The range of electrolyte levels was wide and indicates that the patients with arterial hypertension formed a very heterogeneous group. Four patients in this group had high electrolyte and water contents but did not show any clinical signs of cardiac decompensation. The mean values for these patients were 0 57 mEq Na 0.32 mEq K, and 3 16 g water These values greatly deviate from those for the normal group' the difference is greater than two sigma. The high Na content is especially noteable

Liver tissue from the cirrhosis patients (n = 6) had a mean Na content of 0.32 mEq somewhat more than the normal group The other results were much the same as for the normal group (table I)

Chlotride® treatment resulted in an almost significant reduction in the water content and a significant reduction in both the Na and K levels in the liver tissue (table II) In the placebo group, the Na level dropped probably significantly but there were no changes in the water or K content. There was a mean reduction of 5 4 kg in the body weight of the chlotride group and a mean reduction of 0 4

Table I. The sodium, potassium, and water content of liver tissue obtained by biopsy. Mean values calculated for each group to 95 % limit of confidence

	Normal	Oedema	Hypertension	Cirrhosis
Na	0.22 ± 0.05	0.37 ± 0.13	0.34 ± 0.15	0.32 ± 0.15
K	0.21 ± 0.06	0.28 ± 0.04	0.25 ± 0.08	0.23 ± 0.06
Na/K	1.11 ± 0.32	1.32 ± 0.36	1.50 ± 0.66	1.35 ± 0.51
H_2O	2.11 ± 0.64	3.70 ± 0.89	2.73 ± 0.70	2.42 ± 0.90
$Na + K/H_2O$	0.212 ± 0.035	0.177 ± 0.138	0.222 ± 0.051	0.232 ± 0.074

Table II. The electrolyte and water content of liver biopsy tissue before and after treatment for 14 days with Chlotride® or placebo. Patients selected at random from the groups listed in table I (mean values)

	H_2O	Na	K	Na/K	Body weight (kg)
Chlotride					
Before	2.84	0.31	0.23	1.33	71.3
After	2.06	0.17	0.18	0.93	65.9
Significance					
Placebo					
Before	2.42	0.23	0.20	1.14	68.7
After	2.29	0.17	0.20	0.83	68.5
Significance	—		—		—

= 0.01 < P < 0.05, probably significant.
= 0.001 < P < 0.01 significant.

Table III. Mean change in electrolyte and water content of liver tissue before and after treatment for 14 days with chlotride (= 7) or placebo (= 9)

	Mean change		t-value for difference	P
	Chlotride group	Placebo group		
H_2O	0.78	−0.14	1.77	P = 0.05, probably significant
Na	−0.14	−0.07	1.62	0.05 < P < 0.10 possibly significant
K	−0.05	0	2.02	0.01 < P < 0.05 probably significant
Na/K	−0.40	−0.31	0.56	P = 0.10 not significant
Kg	−3.44	−0.33	2.81	0.001 < P < 0.01 significant

kg for the placebo group. Serum electrolyte levels were relatively constant in both groups.

Comparison of the chlotride and placebo groups can be based on the statistical significance of the changes in the mean values. The placebo group had a mean change in Na level of 0.06 mEq and the corresponding change for the chlotride group was 0.14 mEq, i. e. twice as much.

The difference obtained is between 5 and 10 % and is possibly significant. There was a probably significantly greater reduction in the levels of water and K for the chlotride group compared with the placebo group The reduction in body weight was significantly greater for the chlotride groups (table III)

Discussion

These studies were carried out on relatively few patients and were intended simply as a guide to the differences in the levels of electrolytes and water in the liver tissue of patients with different diseases The liver tissue from all groups contained higher levels of electrolytes than have been reported for other tissues (3) The results of the electrolyte and water determinations on liver tissue from patients with cardiac decompensation have both pathophysiological and diagnostic interest *The levels of Na, K and water were in*creased for this group and distinguished it from the normal hypertensive and cirrhosis groups. Determination of water and electrolyte content in liver tissue can establish whether hepatic oedema is present and also its degree.

Determinations of the electrolyte and water content of liver tissue also afford a possibility for evaluating the efficacy of a diuretic. Treatment of patients taken at random from the different groups gave a probably significant reduction of electrolyte and water levels for the chlotride group and probably significant changes in the Na content and Na/K ratio for the placebo group When the mean changes are compared the drop in Na content for the chlotride group was possibly significant in K and water contents probably significant, and in body weights significant. Chlotride had then, a greater effect upon the electrolyte and water levels in liver tissue but a degree of "hospitalisation effect" was evident in the placebo group. The patients were few and heterogeneous it would be valuable to study the effect of chlotride on liver tissue from a larger and homogeneous group of patients with clinical cardiac decompensation.

Summary

The sodium potassium and water content of liver tissue has been studied on 29 patients divided into four groups normal, hypertensive, oedema and cirrhosis without cardiac decompensation or hypertension The water and sodium content in the liver tissue from patients in the oedema group was significantly higher than in the normal group and the potassium content possibly significantly higher

The hypertensive group was very heterogenous. Of the 12 hypertensive patients four had a significantly increased amount of sodium. Potassium and water levels were slightly increased in comparison with the normal group

In the liver cirrhosis group a slight increase of sodium without significant simultaneous increase of potassium and water levels has been found

A study of the effect of a chlorothiazide diuretic (chlotride®) has also been performed. There was a significant decrease in body weight almost significant decrease in water and potassium and possibly significant decrease in sodium compared with a placebo-group

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The difference obtained is between 5 and 10 % and is possibly significant There was a probably significantly greater reduction in the levels of water and K for the chlotride group compared with the placebo group The reduction in body weight was significantly greater for the chlotride groups (table III)

Discussion

These studies were carried out on relatively few patients and were intended simply as a guide to the differences in the levels of electrolytes and water in the liver tissue of patients with different diseases. The liver tissue from all groups contained higher levels of electrolytes than have been reported for other tissues (3) The results of the electrolyte and water determinations on liver tissue from patients with cardiac decompensation have both pathophysiological and diagnostic interest The levels of Na K, and water were increased for this group and distinguished it from the normal hypertensive and cirrhosis groups Determination of water and electrolyte content in liver tissue can establish whether hepatic oedema is present and also its degree

Determinations of the electrolyte and water content of liver tissue also afford a possibility for evaluating the efficacy of a diuretic. Treatment of patients taken at random from the different groups gave a probably significant reduction of electrolyte and water levels for the chlotride group and probably significant changes in the Na content and Na/K ratio for the placebo group. When the mean changes are compared the drop in Na content for the chlotride group was possibly significant in K and water contents probably significant and in body weights significant Chlotride had then a greater effect upon the electrolyte and water levels in liver tissue but a degree of "hospitalisation effect was evident in the placebo group. The patients were few and heterogeneous it would be valuable to study the effect of chlotride on liver tissue from a larger and homogeneous group of patients with clinical cardiac decompensation.

Summary

The sodium potassium and water content of liver tissue has been studied on 29 patients divided into four groups normal hypertensive oedema and cirrhosis without cardiac decompensation or hypertension The water and sodium content in the liver tissue from patients in the oedema group was significantly higher than in the normal group and the potassium content possibly significantly higher

The hypertensive group was very heterogenous. Of the 12 hypertensive patients four had a significantly increased amount of sodium Potassium and water levels were slightly increased in comparison with the normal group

In the liver cirrhosis group a slight increase of sodium without significant simultaneous increase of potassium and water levels has been found.

A study of the effect of a chlorothiazide diuretic (chlotride®) has also been performed. There was a significant decrease in body weight almost significant decrease in water and potassium and possibly significant decrease in sodium compared with a placebo-group

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Acta Medica Scandinavica. Vol. 175 fasc. 3, 1964

From the Central Laboratory (Head A. Svendsen, mag. scient., M. D.) Central Hospital, Randers, Denmark

Fat Concentration in Faeces

I. Determination of Faecal Fat

By

Karen Johanne Gravesen[1]

Faecal fat may originate from unabsorbed dietary fat, fat from secretions such as the bile or intestinal juices or re-excreted fat from blood or chyle or it may originate from the wear and tear of intestinal mucosa, from bacteria or bacterial synthesis.

Unabsorbed food fat. In recent years Frazer et al. (13-15) have maintained that faecal fat is largely due to unabsorbed food residues. Wollaeger et al. (33) emphasized that faecal fat in the healthy subject is not entirely derived from the fat in the food, although later they expressed the opinion that most of the faecal fat content is determined by the amount of fat ingested. From studies in healthy individuals Annegers et al. (2) concluded that faecal fat is not a residue from dietary fat.

Bile, intestinal mucosa, bacteria. Sperry and Angevine (30) found that faecal fat in dogs did not originate from food or bile nor to any great extent from shreds of intestinal mucosa. Bacterial synthesis could only represent maximally 40 % and probably a much smaller amount, of faecal fat. They proposed an actual excretion into the small intestine with later reabsorption distally either in the small intestine or in the colon. After many studies on dogs and humans Diaz et al. (8) suggested a small fatty excretion into the distal parts of the small intestine of healthy subjects, such excretion being increased in the case of sprue or similar enteropathy. Fowweather (10) believed that fat excretion might take place in the human colon. Finally van de Kamer and Weijers (22) postulated an actual excretion of fat from the blood, chyle or bile into the intestines in coeliac patients. From studies on healthy subjects Frazer et al. (14) suggested that fat could arise from cell breakdown and by secretion.

Chemical composition of fats. Early work on the chemical composition of food fat and its possible influence on faecal fat content has shown that, in children, saturated, long-chained fatty acids re

Submitted for publication July 30, 1963.

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The First International Congress of Parasitology will be held in the Città Universitaria, Roma Italy from 21 to 26 September 1964

All the Parasitologists interested in participating in the congress are requested to send the registration form and the title of their eventual papers to "Segreteria del Primo Congresso Internazionale di Parassitologia, c/o Istituto di Parassitologia, Città Universitaria, Roma, Italy as soon as possible and not later than April 30th, 1964

Congrès International de la Goutte et de la Lithiase Urique Evian France, 4 5 et 6 Septembre 1964

Président: Professeur S. de Sèze.

Secrétariat scientifique. Docteur R. J Réveillaud 4 Boulevard de la Bastille, Paris (12e)

Secrétariat administratif Congrès de la Goutte, Société des Eaux d Evian Evian (Hte-Savoie) France.

LITERATURE ON QUANTITATIVE CHEMICAL DETERMINATION OF FAECAL FAT

1. Conditions of test

Because of the very difficult and time-consuming methods used, only a few studies of faecal fat content were made in the early decades of this century. Early workers disagreed on the question of the fat intake during the test, the periods for which faeces should be collected, and the way of expressing results.

Meals. Fowweather studied 80 patients with no known gastrointestinal disease, eating the ordinary hospital diet, and treated the results as "normal". Most other studies were set up with stricter conditions. Bauer (3) compared the faecal fat content of a healthy two-and-a-half-year-old child with that from a child of about the same age suffering from coeliac disease. These determinations were made on a fat-free diet and after addition of 5 ml cream. Children have been examined on a fat-free diet and after addition of specified fats (19). Krakower (23) studied 12 healthy subjects aged 15 to 60 on four different intakes of food while varying the kind and amount of fat. Eight of the subjects were tried on 4 different fat levels. Wollaeger et al. reviewed earlier studies, then found it necessary to examine the faecal fat content in humans on a fixed and high intake of fat. They first studied 6 healthy subjects as well as 7 patients with duodenal ulcer in a symptom-free period. The diet contained 208 g of fat, largely from milk (33). Later this intake of fat was considered too high for use in patients with steatorrhoea, and subsequently 11 healthy individuals were examined on a diet of 102 g of fat containing the same amounts of carbohydrate and nitrogen as the food given during the first studies. Polak and Pontes (24) used diets providing a daily intake of 109 g of fat for 39 patients and 10 healthy subjects. Annegers et al. (2) were the first to carry out a large series of studies using isocaloric diets and varying the amount of fat. They studied 40 healthy subjects for a week each subject took a total of 93, 140, or 168 g of fat daily. Most subjects were tested on each of these levels of fat intake, and the fat given was from two sources. The 6-week study included 220 experimental periods. This study is more comprehensive, detailed and well controlled than any other fat balance study reported. The conclusions seem pertinent and will be cited later.

Collection of samples. When fat intake is measured the collection periods are specified in number and duration. In order to make sure that the faeces are derived from the period with specified food intake, collection is delayed several days. Carmine markers may be used and enemas may be given before or at the end of a collection. A collection period of several days is considered necessary in most studies. No limitation of the length of the collection period is necessary in order to obtain accurate results of fat in percentage of dry weight. Although the faecal excretion in 24 hours need not be determined, the sample must be a reasonably large one, at least 50 g, to ensure that it is representative.

Storage. Storage conditions before analysis are seldom mentioned. Polak and Pontes (24) found that faeces could be kept for 4 days in an ordinary refrigerator without alteration of the total amount of fat, whether the sample originated from a patient with normal faecal fat content or from a patient with steatorrhoea. Andersen (1) investigated the effect on determination of neutral fat of storing faeces. The basic values were determined at the end of 3-day collection periods, after which the faeces were stored for two days in a refrigerator. After two days' storage faeces from patients with pancreatic steatorrhoea showed a decrease in neutral fat. No normal faeces were examined in this way. She suggested that the decrease in neutral fat level observed might be the result of bacterial hydrolysis of the neutral fat.

The simple way of obtaining samples, using just a small portion of faeces on any ordinary type of food, seemed of considerable interest. The present author therefore, sought to find if the faecal fat from certain patients would differ significantly from that of normal subjects. Thus it was necessary first to examine a considerable number of healthy individuals. It also seemed desirable to check the report of a decrease in neutral fat during storage.

2. Methods of analysis

Early methods were based on the extraction of fat from a sample of faeces, first dried by heating, then powdered (5, 10, 18). A more useful method which involves extraction from the fresh, undried faeces was described by Saxon in 1914 (29). Fowweather carefully

less completely absorbed than shorter unsaturated fatty acids (19) van de Kamer and Weijers (22) confirmed that unsaturated fats were more completely absorbed by using olive oil in studies of coeliac children.

In rats is has been found (9) that natural and synthetic fats with an uneven number of carbon atoms were absorbed at half the rate of even numbered fats.

Radioactivity studies Recent isotopic studies support the concept that the food fat in healthy subjects is usually well absorbed, and that only a small amount of labelled fat appears in faeces Blomstrand (4) using labelled oleic acid found only 0 6 % in faeces from 2 adults Sanders et al. (27) gave labelled glycerol trioleate and obtained the same results in tests on 24 healthy subjects However in 9 healthy subjects Sandweiss and Levy (28) found 5 4 % recovery in faeces of administered oleic acid Radioactivity studies by Ruffin et al. (26) in sprue patients and other gastrointestinal diseases indicate that a content in faeces of below 2 % of ingested labelled oleic acid may be considered normal.

Total faecal fat A routine analysis of faeces includes determination of the fatty acids, and possibly the neutral fat. The total fat includes the sterols, because they are part of the total ether-soluble substances determined by weighing van de Kamer et al (21) uses the expression "total fat content to designate the total amount which is found by titration Fowweather (10) examined the total unsaponifiable matter extracted from 12 samples this consisted mainly of sterols He found values from 0 64 to 3 88 ° of the dry weight. Sterols are not titratable by the methods used

Fatty acids Generally fatty acids make up the greater part of the faecal fat, in the form of free fatty acids, or as neutral fats and soaps.

Neutral fat The neutral fats have been regarded as undigested food fat and have been thought to be of particular significance in the evaluation of the pancreatic function Pratt (25) presents figures from various observations particularly regarding steatorrhoea of pancreatic origin, and concludes that the percentage of neutral fat in the stool is of no aid in the diagnosis of pancreatic or other gastrointestinal diseases In patients with pancreatitis Cooke et al (6) found normal intestinal splitting of fat in 7 cases. Andersen (1) stated "experience has shown that patients with pancreatic deficiency and absence of pancreatic lipase from the duodenal juice may have fatty stools which on analysis give low values for neutral fat. The determination of neutral fat may be of occasional but limited value The fallacy of using the amount of neutral faecal fat as a measurement of pancreatic function should be noted Neither in judging the pancreatic function nor in evaluating the use of pancreatic enzyme preparations, are neutral fat determinations in faeces of definite value, although they may be suggestive.

Soaps The amount of soap present is unimportant since the determination depends not only on the haphazard presence of metal ions (such as Ca and Mg) in the gastrointestinal tract but also on the acidity According to Fowweather many workers believed that little or no significance was to be attached to the relative proportions of fatty acids to soaps. van de Kamer has accepted this opinion.

Miscellaneous The amounts of sterols and fat-soluble vitamins are usually small and are important only when the total fat is determined by weight.

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Finally the amount of fat may be expressed as percentage of dry weight of faeces.

Faecal fat expressed in grams. On an ordinary hospital diet containing 70 to 90 g of fat per day it has been estimated (7) that the fat excretion in faeces should not exceed an average of 6 g/day for a collection period of 3 days. Care should be taken, however, that the food intake is adequate, since in patients with malabsorption, the amount of fat eaten usually influences the faecal fat content to a large degree. On diets of 80 to 190 g of fat and 13 to 18 g of protein nitrogen, Thaysen (31) suggested a daily fat excretion of 10 to 12 g as a maximum based on a collection period of 4 days. On well regulated diets Wollaeger et al. (33) and Annegers et al. (1) expressed faecal fat content as the daily average for collection periods of 3 or 5 days. Polak used a 4-day period, and also the average of a 12-day period obtained by adding results of three 4-day periods. This latter way he considered the more correct. Others have preferred to express in grams the total for the 4-day periods (12, 25).

Faecal fat excretion related to absorbed fat. The expression of faecal fat as percentage of fat intake has been used for many years. An absorption equal to 95 % means that 5 % of the fat eaten can be accounted for in faeces, and that 95 % is considered absorbed. This figure has been called the coefficient of absorption (C. A.). However in 1922 Hill and Bloor (1) clearly expressed the opinion that feeding experiments were of doubtful value unless attention is paid to the amount and kind of fat which appears in the faeces irrespective of the food. Until quite recently the English group working with Cooke et al. and Frazer have used this method of expression, viz. faecal fat in relation to fat intake, but they now state results as the daily excretion in grams.

van de Kamer uses the term "the sliding mean". The average of the C. A. of 3 consecutive days is the sliding mean pertaining to the second of the 3 days. In this way there is elimination of non-essential variations introduced by irregular defaecation and intestinal passage. Admittedly some variations in the true fat absorption may also be eliminated in this way. However for the study of the influence of variations in the fat of the diet on the faecal fat, van de Kamer found it more statistically sound to compare the sliding means, since with this method possible differences between groups can be ascertained more easily. It should be noted that most of the work concerns the study of coeliac disease; for this reason much of the published material originates from examinations of children. The author has not succeeded in finding any data by the Dutch group concerning faecal fat values in adults except C. A. values (32) fig. 6, in which the conditions of test were not stated. Weijers and van de Kamer (fig. 3) stated neither the children's ages nor the faecal fat content when giving normal C. A. values for children.

Faecal fat as percentage of dry weight of faeces. In using this way of expression Fowweather did not state why he considered this method justified. Single faeces collection is much easier to accomplish than a collection over a fixed period, which necessitates close collaboration with ward nurses and patients. He did not use 24-hour samples. In patients receiving the ordinary hospital diet he believed the amount of dry matter in faeces to be of no importance and proceeded to compare 13 "wet" stools (less than 15 % dry weight) — average fat total content 14.9 % of dry weight — with 16 "dry" stools (more than 26 % dry weight) — average total fat content 19.1 %. He also obtained the values for soaps, neutral fats, and fatty acids, and concluded that the two sets of values agreed very well with the final results of his total series of 84 determinations.

van de Kamer argues that the relative dry weight of faeces varies greatly and thus it is impossible to get any impression of fat absorption. However since some correlation exists in healthy children between the fat expressed as percentage of dry weight and the coefficient of absorption, Weijers and van de Kamer (32) relate this to the fact that the amount of dry weight excreted per 24 hours in normal subjects is practically constant. In subjects with faulty absorption no correlation is found, since the amount of dry weight per 24 hours is thought to be dependent on the nature of the absorptive defect. This may be true, but the occurrence of incorrect conclusions based

compared the two methods and reported that the "dry" method gave lower values than the "wet" method because of a less efficient extraction. On the whole the "dry" method gave less consistent results. Neutral fat was taken as the difference between total fat as determined by weight and the titrated fat. In his thesis van de Kamer used a "wet" method. If neutral fat is not to be determined the sample is heated for 20 min with KOH method A. Then the total content of fatty acid regardless of its source is liberated by adding acid.

Using method B the neutral fat is also determined. As heating, even of short duration will promote the suspension of faeces and the liberation of fatty acids bound in the soaps, a *one-minute heating of faeces is carried out in* an acid medium. It was proved that boiling for one minute was sufficient, even if the sample of faeces is very solid. After cooling alcohol is added and the extraction is carried out with petroleum ether. It was shown that shaking during extraction for one minute sufficed. *van de Kamer found, too, that cations added* in specific amount would promote the separation of the lower layer containing the acid alcohol water and faeces, from the upper layer of petroleum ether which contains the fat. *He therefore added NaCl and amyl alcohol.* Using method A no addition of cations is necessary as potassium ion is present in adequate amount. After extraction, the petroleum ether is evaporated and the residue dissolved in alcohol. However the evaporation is not absolutely necessary if only the total fat is to be determined. Then a titration of the fatty acids is carried out preferably with 0.1 N KOH in isobutanol otherwise with 0.1 N KOH in ethanol. When the neutral fat is to be determined also, an extra, known amount of the alkali is added at the end of the first titration then saponification of the neutral fat is carried out by heating. A second titration with acid will give the amount of KOH not neutralized in the saponification. *By subtracting the amount of the unused KOH* from the amount added for saponification, it is possible to calculate the amount of KOH combined with the acids liberated from the neutral fats during the saponification.

Calculations of the amount of faecal fat from titration of sample. Grams of free fatty acids/100 g of faeces is equal to

$$\frac{\text{ml } 0.100\,N\,\text{KOH used} \times 284 \times 1.04 \times 2 \times 100}{\text{g of sample titrated} \times 10 \times 1{,}000}\ \text{g} = \frac{5.907 \times \text{ml KOH}}{\text{g of sample}}$$

The corresponding figure for neutral fat is 5.999. A 0.100 N solution of KOH is equivalent to 1/10th mEq/ml used, and thus the volume of 0.100 N alkali must be divided by 10 to give the corresponding content of mEq acids.

From distillations of fatty acids from human normal faeces van de Kamer (90) found an average molecular weight of 284 for free fatty acids and 297 for acids from neutral fats. 284 mg is equivalent to one mEq acid and must be divided by 1000 to be expressed in *grams. The calculation furthermore includes* using a factor to compensate for loss of acids in the alcohol layer through extraction as well as for the expansion of petroleum ether. The factors are 1.04 for free fatty acids and 1.01 for neutral fats. The number 2 compensates for extraction with 50 ml petroleum ether, *only 25 ml of which are used for titration*, and finally the ml alkali used must be divided by the amount of faeces weighed for titration and multiplied by 100 to express the result per 100 g faeces.

For simplified calculation use the following

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Method B. The sum of free fatty acids and neutral fat.

Result expressed as percentage of dry weight

$$\frac{\text{g of fat/100 g faeces} \times 100}{\text{dry weight/100 g faeces}}$$

3. Ways of expressing results

If a quantitative determination is made, the faecal fat content may be stated in grams of faecal fat during the collection period or per day. In subjects maintained on a diet

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Method B. The sum of free fatty acids and neutral fat.

Result expressed as percentage of dry weight

$$\frac{\text{g of fat/100 g faeces} \times 100}{\text{dry weight/100 g faeces}}\ \%$$

3. *Ways of expressing results*

If a quantitative determination is made, the faecal fat content may be stated in grams of faecal fat during the collection period or per day. In subjects maintained on a diet

in the total amount weighed, giving a higher value for neutral fats than is actually correct. It has been suggested (2) that in Wollaeger's first series with a fat intake of 203 g, gastrointestinal tolerance to fat may have been exceeded — this series comprising 6 healthy subjects and 7 patients with duodenal ulcer in a symptom free period. Other results (2) showed variations similar to those of Wollaeger's second series, the range was from 0.8 to 10.4 g of fat excreted per day (average of 5 days) in 220 experiments. Examination of the individual figures of the various series substantiates the opinion of Annegers et al. which is that highly significant differences exist between subjects. They also stated that neither the quantity nor the type of ingested fat influences the amount of faecal fat excreted in healthy subjects. It thus seems likely that for estimation of the degree of steatorrhoea it will be valid to examine reasonably large sample of faeces, at least 50 grams, from patients eating an ordinary diet in unlimited amount. Results must be expressed as percentage of dry weight in faeces.

OWN INVESTIGATIONS

Material

No previous studies have been published in which there was long-term measurement of faecal fat of healthy individual expressed as percentage of the dry weight. Daily excretion has been calculated as an average obtained from observations covering a certain period. Even if daily determinations have been made, there is lack of figures for individual days as is the case in the studies by van de Kamer and collaborators. Therefore, each of 10 healthy subjects was studied for 10 consecutive days or for 10 days within couple of weeks (one exception (K. V.) had boil on her neck with temperature rise for 2 days just before starting the stud). Of 33 subjects, each examined once, some were healthy subjects, some patients from home for the aged. Of these aged persons some had varicose ulcers and/or suffered from senility. Some patients were from home for the chronic mentally diseased. None of these persons had gastrointestinal complaints, none used laxatives and none had febrile or acute illnesses.

Methods

1 Tests

Meals. All subjects were allowed unlimited amounts and types of food and fats and consumed their usual diet without regulations of any sort.

Collection of samples. A reasonably large sample of faeces was obtained and carried to the author in a waxed carton. Most samples were examined when they arrived at the laboratory but otherwise the sample was stored at 4—6° C until analysis was done. Tests were made to check the permissibility of keeping the samples refrigerated without any further measure of preservation (tables IV and V).

Fat determined. Of primary interest was the total amount of fat. Very few figures are available for the actual amounts of the neutral fat in faeces determined by direct titration. It was impossible to find such information expressed as percentage of faecal dry weight in faeces. The author therefore, decided to determine the normal range of neutral fat under the conditions stated. The total fat then is the sum of the titrated fatty acids plus the neutral fat (van de Kamer method B). However in three cases, BH, KV and GM using 10 samples from each person, only the total fat was determined (van de Kamer method A). Since extraction was made from the undried faeces, special sample from each subject was weighed and dried to determine the faecal dry matter.

Homogenization. For mixing the sample of faeces van de Kamer used mortar. Since the faeces was carried to the laboratory in waxed and rather stiff container it was decided to mix the contents in the container itself by using spatula. The remainder after the analytical specimen has been weighed ca thus be disposed of with the container.

Weighing of the samples. The weighing should be done as quickly as possible t prevent evaporation during weighing. About 5 g of faeces should be used for each determination, with duplicate determinations made for drying the specimens as well as for the fat determination. If large amounts of fat ar expected, an amount smaller than 5 g should be used for the actual analysis.

Determination of dry weight. About 5 g of well mixed faeces are smeared on the sides of a tared glass weighing vessel with light-fitting

Table I Normal' daily fat excretion

Authors	No. of exp.	Days	Fat eaten excreted (g)	% of dry weight		Method
				Total fat	Neutral fat	
Fowweather (10)	84	—	—	—	—	
				7.3–27.8	2.59–11.80	Saxon
Average				17.5	7.51	
Wollaeger et al. (33)	13	3	208	—	—	Saxon
			5.5–13.6	27.5–46.8		
Average			8.7	33.5		
Wollaeger et al. (34)	11	3	106	—	—	—
			1.8–6.7	9.3–19.6		
Average			4.1	14.5		
Polak & Pontes (24)	10	12	109	—	—	van de Kamer
			1.69–5.33			
Average			3.58			
Annegers et al. (2)	40	5	93	—	—	Saxon modified and Fowweather Anderse
Average			4.33			
SD			1.9			
	40	5	93	—	—	
Average			3.75			
SD			1.5			
	30	5	140	—	—	
Average			3.60			
SD			1.4			
	30	5	140	—	—	
Average			3.90			
SD			1.0			
	40	5	168	—	—	
Average			3.75			
SD			1.9			
	40	5	168	—	—	
Average			4.10			
SD			1.5			

on faecal fat percentage in those cases can be checked through clinical observation and inspection of the amount of faeces.

4 Some earlier results (6, 12 33)

Table I shows the most important comprehensive studies in recent years and includes the earlier results of Fowweather

Fowweather examined 84 samples from 40 males and 40 females. In his results the values found for neutral fat should be noted. When using van de Kamer's method the present author found low neutral fat values in comparison with the figures in the literature (10, 16). The reason for the higher figures in Fowweather's results is that neutral fat is determined as the difference between fatty acids, titrated, and total fat when weighed. This is not evident from his first papers, but Fowweather et al explicitly stated later that the neutral fats were determined in this way Thus all ether-extractable lipids are included

Table II. Errors in duplicate determinations of 10 consecutive samples (case G G)

Dry weight (%)		Total fat (g/100 g)			
		Wet weight basis		Dry weight basis	
	Average		Average		Average
27.33	27.89 ± 2.0%	2.582	2.530 ± 2.1%	9.26	9.07 ± 2.1%
28.45		2.477		8.88	
24.59	24.68 ± 0.4%	1.663	1.709 ± 2.7%	6.74	6.92 ± 2.6%
24.78		1.755		7.11	
23.74	23.24 ± 2.2%	2.023	2.030 ± 0.3%	8.70	8.73 ± 0.3%
22.75		2.037		8.77	
20.83	20.74 ± 0.5%	1.933	1.763 ± 9.8%	9.33	8.[illegible]0 ± 9.8%
20.65		1.592		7.68	
23.42	24.72 ± 5.5%	2.417	2.294 ± 5.4	9.78	9.28 ± 5.4%
26.02		2.171		8.78	
25.62	26.40 ± 3.0%	2.181	2.242 ± 2.8%	8.26	8.50 ± 2.1
27.18		2.304		8.73	
19.22	19.46 ± 1.2%	2.045	1.887 ± 8.3	10.51	9.70 ± 8.4
19.71		1.730		8.89	
18.39	18.70 ± 1.7%	1.421	1.428 ± 0.5	7.60	7.64 ± 0.5
19.02		1.436		7.68	
21.42	21.77 ± 1.6	1.472	1.527 ± 3.7%	6.78	7.01 ± 3.6
22.13		1.583		7.27	
23.32	23.45 ± 0.5	1.945	1.789 ± 8.1	8.29	7.67 ± 8.1%
23.57		1.633		7.05	
		Average error	± 4.3		
				Mean fat content	8.30%
				SD	0.96
				SD%	11.6

I the calculation of the error tw decimals in the individual figures were considered.

vigorously for 1 min. The tube is now placed standing in the rack for separation, which may be hastened by gentle rotation. It is not necessary to add amyl alcohol. After separation, 25 ml of the extract is transferred to 50 ml Erlenmeyer flask.

Evaporation. Two tiny pieces of soft paper are added to prevent splashing (pumice should not be used). The flasks are placed on the metal lid of waterbath well bov the surface of the boiling water. When dry or nearly dry the residue is dissolved in 2 or 3 ml neutralized ethanol in order to make the titration easier and to keep the indicator 2 or 3 drops of phenolphthalein, dissolved.

Th 98 % ethanol used had negligibl acidity as 5 ml could be neutralized with as little as 0.010 to 0.015 ml of the alkali, used for titration. Since 3 to 6 ml alkali are used for titration of most faeces samples, any error due to use of non-neutral ethanol would be trivial.

Titration. Before titration of the sample the normality of the KOH in isobutanol must be determined through titration with 0.1000 N HCl. It is important to use some ethanol

lid. In order to obtain the exact amount of faeces, the vessel is weighed with the lid on, the lid is then removed and the drying is carried out for two hours at 110 C. Longer drying is unnecessary since changes will usually be seen only in the third decimal when the result is reported in grams. The lid is replaced and after spontaneous cooling for 30 min., or longer the glass is weighed with the lid on to obtain the dry weight of the sample of faeces.

$\frac{\text{Dry weight} \times 100}{\text{wet weight}}$ is equivalent to the percentage of dry matter in faeces.

Several millilitres of alkali, e. g 0.5 N KOH or NaOH are poured into the vessel, and it is left until the next day when the faeces, usually as a hard cake can easily be removed.

Equipment for analysis. Glass tubes for extraction were made of 50 ml Kjeldahl bulbs. A B 34 socket was added at the top to fit the condenser At the top on the outside were two hooks on which rubber bands were fixed to attach the condenser

As van de Kamer recommended, a pipette inserted in a rubber stopper of suitable size to fit the extraction tube was used for removal of petroleum ether following separation of extracts. The use of a Kjeldahl bulb instead of an Erlenmeyer flask greatly facilitated the removal. Ordinary 50 ml Erlenmeyer flat bottomed flasks fitting a condenser were used for titration and saponification.

5 ml burettes were used for titration.

Reagents The water used and termed H_2O below was de-mineralized.

1 33 % KOH. 150 g KOH dissolved in 300 ml H_2O

2 25 % HCl. 658 ml concentrated HCl diluted to 1 000 ml with H_2O

3 van de Kamer's acid salt-solution 750 g NaCl dissolved in about 900 ml H_2O 70 ml concentrated HCl is added and the solution is diluted to 1 000 ml with H_2O

4 98 % ethanol.

5 Neutralized ethanol is obtained with phenolphthalein as an indicator by adding small amounts of weak NaOH to 98 % ethanol until the colour turns slightly pink.

6 Petroleum ether reagent grade, boiling at 40—60° C. Titration of residue from petroleum ether dissolved in ethanol showed no acid substance.

7 0 1000 N HCl prepared by dilution from constantly boiling HCl.

8. Approximately 0.1 N KOH in isobutanol was prepared. The theoretical amount of KOH necessary for making a 0.1 N solution would be 5 6 g, but due to the difficulty in transferring the aqueous solution of KOH, a larger amount proved necessary It was unnecessary to leave the KOH standing after the pellets had been dissolved in H_2O Instead of using a 50 % solution of KOH as taken by van de Kamer the author used 7 g/l of KOH of isobutanol, dissolving the KOH in a small beaker in 4 ml H_2O and adding 3 or 4 ml methanol for transfer The beaker was rinsed twice with 2 or 3 ml methanol. At the beginning of the daily titration, the exact normality of KOH was determined, but as the solution keeps well after a day or two of stabilization, this titration need only be done occasionally The reagent was kept in a brown bottle

9 Tiny pieces of soft paper giving no titratable values were left in petroleum ether during evaporation.

10 Phenolphthalein indicator 0.1 % in 98 ethanol was used.

2 Method B

This is the method used for analysis of all specimens with the exception of those from subjects HB, KV and GM. In method B free fatty acids as well as neutral fats are determined by titration the sum of the two determinations gives the total amount of fat.

Extraction. About 5 g of well mixed faeces is weighed into the modified Kjeldahl bulb, 22 ml of acid NaCl solution is added from a graduated cylinder and the condenser attached. The tube may be left for treatment later on or may be heated at once. The mixture is kept boiling for 1 min. with the condenser on, then the flask is placed in a cold water bath (approximately 15—18 C) where the water is changed constantly The condenser is left attached to the glass tube After about 20 min 20 ml 98 alcohol is added from a graduated cylinder and after the water-bath cooling for another 20 min., the tube is placed in a suitable rack and the condenser is removed 50 ml petroleum ether is added with a pipette. A glass stopper is used for closing the tube, which is then shaken

Table V. Importance of amounts of petroleum ether extract used

Case	Dry weight (% of faeces)	Neutral fat (g/100 g)				Total fat (g/100 g)			
		Wet weight basis		Dry weight basis		Wet weight basis		Dry weight basis	
		25 ml	12.5 ml	25 ml	12.5 ml	25 ml	12.5 ml	25 ml	12.5 ml
C. M.	27.61 (12.5 ml kept one day)	0.308	0.350	1.11	1.27	2.477	2.347	8.79	9.21
G. M.	29.58	–	–	–	–	4.96	5.05	16.8	17.1
G. M.	16.70	–	–	–	–	2.14	2.32	12.8	13.9
G. M.	18.18	–	–	–	–	2.51	2.72	13.8	15.0
	Single determination								
C. M.	23.24	0.182	0.190	0.78	0.82	1.80	1.88	7.74	8.07

Saponification and second titration. When the free fatty acids have been neutralized as above, an extra amount of 2.5 ml KOH in isobutanol is added, a condenser attached to the flask, and the content of the flask kept gently boiling for 30 min. during which time the neutral fats are split into glycerol and free fatty acids. After boiling, while the flask is still warm, 10 ml neutralized ethanol is added, and the remaining alkalinity is determined by immediate titration with 0.1000 N HCl. According to van de Kamer the liberated acids, if left to cool, may combine again with the glycerol then a smaller amount of the alkali added for saponification is conjugated with the acids from the neutral fats. Consequently more is left to be titrated as "unused" giving a lower value for neutral fat. The titration is carried to a constant yellow end-point. From the amount of acid used, the amount of KOH neutralized by acids liberated during boiling is easily calculated. Thus subtracting the amount of KOH neutralized by HCl from the amount of KOH added for saponification, the amount used by the neutral fat is found. Example: 2.5 ml 0.106 N KOH in isobutanol is added for saponification corresponding to 0.106 × 2.5 ml 0.100 N which is equivalent to 2.65 ml. After saponification 1.43 ml 0.1000 N HCl is used for titration leaving (2.65 minus 1.43) ml 0.100 N KOH which indicates the amount of neutral fat as equivalents of fatty acids.

3. Method A

Only total fat is determined, the method giving the same value for total fat as method B.

Extraction. After the sample of about 5 g has been weighed into the tube, 10 ml 33 % KOH is added from a small graduated cylinder and 40 ml ethanol is added. The condenser is connected and the mixture allowed to boil for 20 min. Then 17 ml 25 % HCl is added from a graduated cylinder after a cooling period of 20 min., and cooling continued for another 20 min. after which the analysis is continued as explained in method B.

Titration. The dissolved residue is titrated with KOH in isobutanol. This titration covers the total fats, i. e. the acids whether originally free, bound in soaps, or as neutral fat. For any further details regarding fat analyses the reader is referred to van de Kamer's thesis.

4. Duplicate determinations

In his thesis van de Kamer tabulates the agreement between duplicates in his own method as compared with other methods. This was within a few per cent, with use of a mortar for homogenization.

Table II shows the daily variation between duplicates in routine analyses, in an individual with a fairly low percentage of faecal fat. The table further presents the daily variation of his faecal fat content. Other determinations — duplicates of 10 samples from each of 5 sub-

Table III Storage values total fat in g/100 g of wet faeces and total fat in percentage of dry weight of faeces

Case	Date	Faecal dry weight (g/100 g faeces)		Total fat (g/100 g)			
				Wet weight basis		Dry weight basis	
			Average		Average		Average
H. B.	12.2	28.94 28.17	28.55 ± 1.3%	[illegible].759 2.879	2.82 ± 2.1%	9.87	10.06 ± 1.9%
	13.2	28.94		2.863 2.991	2.93 ± 2.2%	10.25	
G. M.	29.1	23.93 24.30	24.11 ± 0.8%	1.405 1.591	1.50 ± 6.2%	6.17	6.53 ± 5.5%
	1.2	24.26 24.41	24.33 ± 0.3%	1.679 1.675	1.68 ± 0.1%	6.89	
G. M.	18.1	17.34 16.07	16.70 ± 3.8%	2.079 2.204	2.14 ± 2.9%	12.81	12.60 ± 1.7%
	20.1	17.47 17.37	17.42 ± 0.3%	2.107 2.203	[illegible].16 ± 2.2%	12.40	
C. G.	4.1	23.86 23.04	23.45 ± 1.7%	4.104 3.993	4.05 ± 1.4%	17.26	17.35 ± 0.5%
	8.1	25.08 24.74	24.91 ± 0.7%	4.303 4.389	4.45 ± 0.9%	17.45	

In the calculation of the error two decimals in the individual figures were considered.

Table IV Storage values, neutral fat in g/100 g of wet faeces

Case	Date		
C. M.	15.5	Single determination	0.182
	16.5	—	0.[illegible]44
K. N.	10.8	0.329 0.201	0.265 (average)
	12.8	0.275 0.251	0.263
C. M.	First day	0.273 0.34	0.308
	Next day	0.358 0.342	0.350 (12.5 ml samples)

(2 – 3 ml to 2 – 5 ml alkali) with the alkali as otherwise the thorough mixing of the alkali and acid is delayed, in which case the conditions established are not similar to those of the titration of the sample.

The redissolved petroleum ether extract is now titrated with KOH. van de Kamer indicates that the end-point of titration needs more careful observation when phenolphthalein is used as an indicator instead of thymol blue although the end points are at almost the same pH. I preferred phenolphthalein and had no difficulty in determining the end point change from yellowish to red dish-brown when the extract is titrated with alkali. The amount of alkali used corresponds to the amount of free fatty acids in the 25 ml extract, whether originally free in faeces or bound as soaps and liberated through the heating with HCl.

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jects — gave average errors of 4 3 4 1 4.8, 5 7 and 3 7 % respectively Although the error in duplicate determinations might be reduced by using mechanical devices for homogenization, the errors found are not really serious.

5 Influence of storage on results

van de Kamer does not mention the influence of keeping faeces for some time before analysis. Table *III* confirms Polak s observation that faeces need not be analysed immediately and also indicates again the error in duplicate determinations. Subjects HB and GM represent method A, and subject CG method B.

Andersen has measured the amounts of neutral fat before and after storage of faeces from patients with pancreatic disorders, but corresponding determinations from healthy subjects have not been published Table IV shows that no definite alteration took place in neutral fat values of faeces from healthy subjects when faeces were stored at 4—6° C. Since only a small amount of neutral fat is found, the percentage error will be higher than for total faecal fat due to titration technique.

In determining the reproducibility of the results it was of interest to check whether a 12.5 ml sample of extract instead of the usual 25 ml would give an acceptable result. If so should one of the duplicate samples be lost, 12.5 ml could still be taken out of the extraction tube

Table V presents the results of duplicat determinations made with 25 ml as well as with 12 5 ml samples, the latter all showing slightly higher values. Although an occasional sample might be determined by using 12 5 ml only such small samples should generally not be used It can be concluded that with 25 ml duplicates for titration the described way of sampling and expressing results is satisfactory for routine clinical use.

Summary

A historical survey is presented of methods for the determination of faecal fat with special emphasis on the conditions prior to collection of the sample and on the methods for expressing results. Some early results are cited.

A description is given of the normal subjects studied and of the method used by the author a random sample of faeces weighing at least 50 g was examined by van de Kamer s method with the subjects on any unlimited ordinary diet.

Results for neutral fat and total fat content are expressed as percentage of faecal dry weight. The reproducibility of the determination is demonstrated Faecal fat values for stored faecal samples are similar to those of fresh specimens. It is concluded that the procedure including the method of expression of results is satisfactory for routine clinical use.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Fat Concentration in Faeces

II. Determinations of Faecal Fat in Healthy Subjects

By

Karen Johanne Gravesen[1]

In a previous article (10) the author described the method for determining and expressing faecal fat in normal subjects after outlining the conditions of the study. The present article deals with the results of studies on 43 normal subjects, and in a paper to be published the results in patients after gastric resection (a.m. Hofmeister) will be reported.

Results

Each of ten healthy subjects was studied for 10 days, while 33 other subjects were examined once. Since the children designated as LP and KN had eaten a great amount of plums a day or two before the examination, the results of determinations relating to these persons are excluded from statistical computations. A total of 133 samples is presented (table I).

The whole group, regardless of age and not excluding LP and KN, is represented by the histogram of 133 samples (fig. 1), the 100 samples from 10 subjects being treated as 100 individual samples.

Individual excretion. Table II of the previous article for faeces from case G. G. showed the errors in duplicate determinations, as well as the mean, the standard deviation (S. D.) and the coefficient of variation (S. D. %). Using probit diagrams the distribution of the 10 total-fat values was examined for each of the 10 subjects and the resulting distribution for each case was accepted as normal.

By means of a "false χ^2-test" the variances for each of the 10 subjects were analysed and they were accepted as being equal. Finally an analysis was made to examine whether the variation between individuals was greater than the variation in the daily excretion of the individual subject (i. e. to find whether the 10 × 10 samples could be considered as 100 individual samples).

If the inter-individual and intra-individual variances were the same the value s_1^2/s_2^2 would follow an F-distribution. The calculated value is significant with respect

Submitted for publication July 30, 1963.

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Table I (cont.)

Case	Age (yrs)	% Dry eight	Fat as % of dry weight		
			FFA	NF	Total
Males					
L. P	6	28.05	10.58	1.56	11.94
S. F	12	30.62	8.45	1.03	9 48
B. F	14	23..9	5.65	1.98	7.64
P R.	33	16.94	4.50	2.30	6.80
E. H. M.	39	22.90	7.54	1.93	9.47
T P	42	29.54	10.32	1 16	11 49
G F	47	31 47	8.18	1.12	9.29
J. P B.	68	35.58	10.67	0.76	11 43
J. J	71	25.13	16.96	1 43	18.39
S. S.	78	19.57	15.01	2.38	17.39
H. F	81	33.28	14 15	1 17	15.32
Females					
K. N	8	33.62	17.23	1.65	19.54
K. O.	11	20.12	4.88	1.09	5.96
E. O.	12	31 49	3.69	0 09	3.78
K. K N.	18	33.40	8.17	0.46	8.62
R. W	22	26.43	8.49	0.82	9.31
K. R N	22	35 55	7 51	0.43	7.94
K P	37	33.62	11.36	1.84	13.20
G. L. J.	37	31.32	7.86	0.11	7 97
S F	48	25.79	7.81	0.40	8.21
M. S.	52	29.23	7 46	0 63	8.08
O C.	54	44.87	3 33	0.64	3.98
G D C	54	33 85	10 37	0..6	10.93
M P F	58	30 86	15.25	0.28	15.53
A. T	62	24 88	16 28	0.30	16.58
E J	62	22 91	13 64	0 34	13 98
P K E	68	34 83	10 32	0.93	11.25
O. A.	69	24 69	11 83	2.11	13.92
M. J	72	26 70	11.09	1 19	12.28
L P	74	29 65	15.85	1 56	17 41
A M N	81	36.59	9.36	0.56	9 92
K. A.	81	28 1	17 55	1 69	18.24
E D	88	25 84	17 50	1 30	18.80
No. of observations			101	101	131
Means			8.16	1.27	10.66
Ranges			3.69–17.55	0.09–2.38	3.78–19.54

Excluded from statistical computations due to excessive plum-eating

From home for the aged.

Mentally deranged.

FFA = free fatty acids NF = neutral fat.

Table II Analysis of variance

Case no.	$\bar{X}$	Σ $(\bar{X}\ X)$	$(\bar{X}-\bar{\bar{X}})^2$ $\times 10$	Degrees of freedom
1 G. G.	8.3	8.34	44.1	–
2. H. H.	6.3	30.83	168.1	–
3. C. M.	9.2	40 89	14 4	–
4. C. G.	11.9	33.82	22.5	–
5. A. S.	14 4	67.23	160.0	–
6. K. V	10.9	113.97	2.5	–
7 H. B.	10 7	75.44	0 9	–
8. G. M.	12.9	98 75	62.5	–
9. K. N.	8 4	41.81	40.0	–
10. J G.	11.0	42.60	3.6	–
Σ	104.0	555.68	–	90 $s_1 = 6.174$
$\bar{X}_0$	10.4	–	–	–
Σ	–	–	318.8	9 $s_2 = 57.62$

to the 0 1 per cent level We must, therefore, conclude that the variation between individuals is greater than the variation in the individual subject. Consequently each group of 10 samples can only be considered to represent an average for that individual, that is as one sample (fig 2) on which is based the standard deviation (S D) Considering the errors in duplicate determinations (tables II and III given in the previous article (10)) one can conclude that both the individual variations and the variations between individuals are of such a magnitude that they can hardly be affected by the errors in duplicate determinations.

Even using samples from a 5-day collection of faeces Annegers et al (1) found results essentially like these.

Sex and age Excluding the figures for L. P aged 6 and K. N aged 8 for reasons stated previously the total fat percentages for 41 subjects are plotted against age in fig. 2. The mean is used for each of the 10 subjects described in table II

Table I Dry weight of faeces and faecal fat 133 samples from 43 subjects

Case no. *Males*	Age (yrs)	% Dry weight	Fats as % of dry weight			Case no. *Females*	Age (yrs)	% Dry weight	Fats as % of dry weight		
			FFA	NF	Total				FFA	NF	Total
1 G G	26	27.89	7.60	1.47	9.07	6. K. V	20	29.28	–	–	10.77
		24.68	5.80	1.12	6.92			30.63	–	–	11.08
		23.24	7.13	1.60	8.73			25.30	–	–	18.63
		20.74	6.92	1.59	8.50			26.87	–	–	7.05
		24.72	7.37	1.70	9.28			26.73	–	–	7.02
		26.40	7.45	1.05	8.49			24.58	–	–	7.25
		19.46	7.89	1.80	9.70			26.58	–	–	9.79
		18.70	5.98	1.66	7.64			31.86	–	–	12.80
		21.77	5.47	1.55	7.01			31.95	–	–	13.11
		23.45	5.86	1.81	7.67			26.27	–	–	11.84
2. H. H	26	24.84	6.73	0.77	7.50	7 H. B	20	29.41	–	–	11.15
		35.65	5.03	0.96	5.99			28.80	–	–	7.85
		36.60	3.79	0.76	4.54			27.18	–	–	5.69
		34.91	8.98	1.21	10.19			21.80	–	–	7.93
		30.07	5.15	1.01	6.16			26.41	–	–	[illegible]
		36.13	4.65	0.87	5.52			26.52	–	–	15.21
		35.83	4.34	0.70	5.04			7.56	–	–	13.82
		37.36	3.82	0.56	4.38			28.21	–	–	12.77
		34.16	5.12	0.50	5.62			28.55	–	–	10.25
		34.62	7.12	1.26	8.38			30.97	–	–	9.98
3. C. M.	33	23.24	6.80	0.95	7.75	8. G. M	23	29.58	–	–	16.77
		30.58	8.64	2.01	10.65			16.70	–	–	12.81
		30.88	4.13	0.59	4.72			18.17	–	–	13.81
		7.61	7.68	1.16	8.84			29.20	–	–	16.88
		30.71	6.62	1.34	7.96			28.81	–	–	13.57
		25.75	8.57	1.42	9.99			23.22	–	–	10.77
		34.00	8.63	1.72	10.35			17.11	–	–	10.17
		19.63	10.42	1.32	11.74			27.28	–	–	15.94
		16.81	7.00	1.18	8.12			24.11	–	–	6.17
		21.60	9.72	1.74	11.46			28.66	–	–	12.07
4 C. G	42	28.50	11.79	1.68	13.47	9 K. N	38	32.78	9.81	1.07	10.89
		24.21	11.73	2.25	13.98			32.90	10.37	1.20	11.57
		22.89	12.41	1.73	14.14			32.54	5.30	0.74	6.04
		17.85	12.46	1.77	14.23			34.35	7.44	0.99	8.43
		22.96	8.38	1.46	9.84			[illegible]	7.65	0.77	8.42
		27.03	8.29	0.92	9.21			31.01	7.89	1.16	9.06
		17.98	8.54	0.86	9.40			35.50	5.10	0.35	5.45
		19.30	9.90	1.70	11.60			30.46	9.01	1.44	10.46
		20.33	9.89	1.77	11.66			35.86	6.83	0.91	7.74
		18.63	9.65	1.46	11.11			34.04	5.16	0.85	6.01
5. A. S.	44	24.95	13.97	2.15	16.12	10. J G	42	35.29	9.22	1.88	11.10
		31.20	16.27	1.71	17.98			33.46	7.80	2.01	9.81
		26.80	8.71	1.66	10.37			31.18	[illegible]	2.76	15.14
		25.50	10.11	1.59	11.70			31.58	11.97	1.65	13.62
		28.33	12.29	1.37	13.66			36.26	11.03	1.10	12.13
		29.89	10.73	1.56	12.29			34.30	9.99	1.60	11.59
		20.55	15.86	1.54	17.40			30.44	6.67	1.54	8.21
		19.64	10.23	1.88	12.11			28.22	8.53	2.06	10.59
		31.40	13.86	1.23	15.09			29.86	8.59	0.90	9.49
		28.99	15.47	1.78	17.25			34.21	7.32	1.23	8.55

Discussion

Comparison of results with other reported studies in normals

van de Kamer et al. (12) express their results as percentage of fat intake. As these uthors have not given any original figures for either normal or pathological values obtained from actual analyses, no comparison can be made.

Annegers et al. (1) used very exact intakes of food with fixed fat levels. The coefficients of variation from the six series of studies (220 experiments) were 43 9 40 0 38.9 25 6, 50.7 and 36 6 %. The ages of the individuals were not given

In the present study the coefficient of variation is 33.6 % regardless of age. Therefore, on a free intake of food with the fat content expressed as percentage of dry weight, the coefficient of variation is even smaller than in the very carefully conducted experiments of Annegers et al.

Bromfield (2) who used the Soxhlet method, stated that the normal fat content generally ranges from 10 to 15 % total fat as percentage of dry weight. H published table of faecal fat values for 20 healthy subjects, 10 patients with tropical sprue and 3 patients suffering from idiopathic steatorrhoea. No information was given about age E idently the fat content of the diet was not fixed. Individual values are not given however the mean for the 20 healthy subjects is 13 1 % total fat range 7 5—17 7) with no mention of standard deviation or other details. These normal alues fall within the range of the present tudy

Fowweather 9) found values widely different from those now found. The mean of his total fat values was 17.5 % 7.3—27 8) and the mean of his neutral fat alues was 7.3 (2.39—11.80) The reason for these higher values is that total fat was determined by weighing, and this also increases the values for neutral fat found by Fowweather

Wollaeger et al. (14 15) (see article I table I (10)) had a first series (14) with subjects on a fat intake later considered too high for use in patients with steatorrhoea. Their second series, of 11 subjects aged 21 to 31 had an average of 14.5 % fat ranging from 9.3 to 19 6 % of dry weight (15) These figures agree closely with Bromfield s and mine. Also the method used is similar to mine.

In a description of metabolic studies of sprue patients at the Mayo clinic, Comfort et al. (4) gave 22.9 % total fat as the upper normal limit (mean plus 2 S D based on (15)) In this study the highest value found was 19 5 %

Comparison of various way of expressing results in patients

The method of expressing results used in this study — fat as percentage of dry weight — may be compared with the two methods used by Cooke et al In addition the method will be evaluated by studying the results of 48 consecuti e analyses of faeces from patients at the Central Hospital, Randers, where 24-hour specimens were collected and analysed and results expressed in g/24 hours as well as in percentage of dry weight.

Cooke et al. (5 6) used the method o Cammidge an ntake of 50 g of fat pe day with collection of faeces for 5 days They regarded coefficient of absorption of less than 90 as bnormal (5) Later with a more liberal diet containing 70 t 90 g of fat per day and with a collectio of approximately 3 days duration the considered a daily fat excretion greate than 6 g as abnormal (7) They now u the method of van de Kamer for fat d

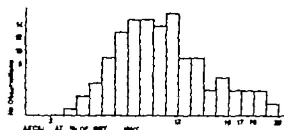


Fig 1 Faecal fat 133 samples. Observations covering e.g faecal fat content of 11—12 % represent values from 11 00 to 11.99 %

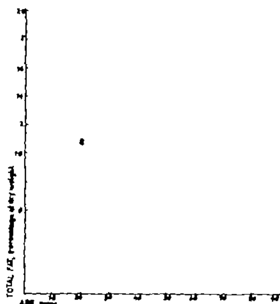


Fig 2 Total fat percentage for 41 subjects in relation to sex and age (males ⊕ females ○)

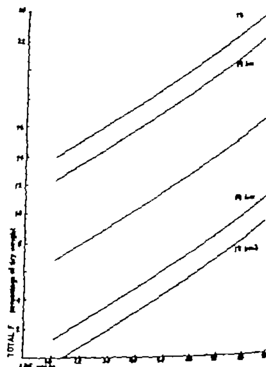


Fig 3 Expected total faecal fat as percentage of dry weight in relation to age

The normal range of excretion A rise in faecal fat content as percentage of dry weight is seen with increasing age Since moreover no sex difference has been observed, the regression line (fig 3) expressed in the following equation

fat percentage = 0 1214 age in years + 5 63

best describes the normal values for faecal fat content as percentage of dry weight Since the subjects with repeated examinations are represented only by the mean value in the calculation of the regression equation 41 individuals form the basis of the calculation.

The standard deviation estimated in the regression calculation is found to be 2 80 thus referring to the average of duplicate tests on samples from a mixed single collection of faeces. The standard deviation multiplied by 2 58 and 1 96 establishes the distance from the regression line of the 99 and the 95 % confidence limits, respectively A graphic representation of confidence limits is shown in fig 3 Evidently 95 % of normal subjects, ingesting an ordinary unlimited diet, would fall within the inner limits, and 99 would fall within the outer limits. The faecal samples should be at least 50 g in weight Generally between the ages 10 and 70 the total faecal fat as percentage of dry weight in 95 % of healthy subjects would be below 20 which is also the highest value found in this study (19 5) At the age of 40 5 % of subjects at the most could be expected to have faecal fat values above 16 % of dry weight

Table IV Analyses of 48 consecutive 24-hour portions of faeces from patients. (Central Hospital, Randers 1957—1959)

Case no.	Age (yrs)	Faeces (g)	% Dry weight	Total fat % of dry weight	Total fat g	Comments
1957						
1,607	53	144	32.4	22.5	10.5	With an interval of 8 days
1,607	53	110	37.2	31.4	12.8	
3,053	55	109	30.4	13.6	7.5	
3,503	59	179	31.0	43.5	25.2	
6,102	59	250	40.0	25.5	25.5	
6,344	73	355	32.1	33.7	38.4	
6,183	76	40	33.6	13.5	1.8	
6,785	49	511	32.4	10.5	17.4	
7,658	59	950	29.0	36.2	99.7	
7,787	37	43	31.4	13.4	1.9	
7,979	61	53	29.5	*23.7	3.7	
1958						
63	50	135	23.8	12.3	4.0	
3,842	49	110	31.9	13.1	7.5	
960	58	210	25.1	28.8	15.2	
1,190	78	165	23.4	38.3	15.2	
1,919	46	319	24.3	31.6	24.5	
1,845	38	146	23.8	20.9	7.9	
2,174	49	168	18.1	16.8	5.4	
2,735	69	88	30.2	11.2	3.8	
3,127	58	485	23.8	31.6	45.7	
3,247	59	86	23.5	19.9	4.0	
3,817	65	131	26.4	20.4	8.1	
4,531	20	151	31.5	2.7	1.3	
5,123	58	112	21.7	44.8	10.9	
4,547	46	145	28.9	33.0	14.3	
4,841	52	109	33.2	18.7	6.8	
5,420	27	100	13.1	10.5	1.4	
5,937	48	195	22.0	30.8	13.2	
7,363	17	143	28.6	16.2	6.6	
7,740	57	96	39.0	52.0	19.5	
7,852	14	60	19.7	8.3	1.0	
1959						
149	56	225	22.4	15.6	7.9	
190	58	117	28.5	51.8	17.3	Three determinations with intervals of 12 days. Note similarity of fat percentages
190	58	600	25.5	49.9	76.3	
190	58	380	27.2	49.8	51.5	
258	64	122	26.5	17.8	3.8	
494	52	233	19.1	22.1	9.9	
1,129	50	52	33.5	22.4	3.9	

Table III Six cases from Cooke et al. (5) with differences in results

Case no. (Cooke)	Age	Total fat excretion		Coefficient of absorption
		g	% of dry weight	
54	48	5.2	26.7	90
56	48	5.5	26.4	69
57	33	[illegible]	36.6	89
79	22	5.3	17.5	69
82	29	6.6	12.9	87
83	21	5.9	21.8	88

termination Their upper limit value of 6 g was based on the studies described in (1) (6) and (15) Furthermore Fourman et al (8) with 5 subjects on a daily fat intake of 70 g showed that an excretion of 23 g over 4 days should be considered as the upper limit (mean plus 3 S D i e. covering 99 8 % of observations made)

Polak and Pontes (13) considered a 4—7 9 g fat excretion per day as borderline value, and Kalser regarded 5—7 g per day as suggestive of malabsorption, above 7 g a definite steatorrhoea.

Comfort (3 4) fixed 7 g as the upper limit (mean plus 2 S D) based on Wollaeger s work (15) and stated that increased fat excretion can be discovered by examining a random sample and expressing the result as percentage of dry weight without the patient being on standardized food intake; however if the chemical fat values found were borderline and on the other hand the stools were not grossly fatty balance tests should be made. The figures published by Cooke et al (5) show the results in patients with idiopathic steatorrhoea in both good and poor condition They comprise a total of 72 experimental periods, most periods with a 48-hour collection, some with collection periods of 72 96 and even 120 hours.

In 11 of the experimental periods the results are judged normal and in 55 cases abnormal according to all three ways of expression i e. results considered abnormal with fat excretion above 6 g fat absorption less than 90 % and fat as percentage of dry weight outside the limits given in the present paper age taken into consideration (fig 3) This must be considered very good agreement.

The remaining 6 cases are presented in table III In 4 of the cases in table III the values are deemed abnormal, whether expressed as percentage of dry weight or as C. A. However a discrepancy exists between the two criteria used by Cooke et al and therefore, also between fat as percentage of dry weight and fat in g/day (cases 56 57 79 and 83) In case 54 the fat percentage is abnormal, while the two other figures are normal and in case 82 the feacal fat percentage is normal total fat excretion in g as well as C. A. being abnormal. It is evident that these 6 cases present borderline values, in which cases examination and expression in various ways may be helpful to establish the diagnosis of steatorrhoea.

The method of expression of faecal fat as percentage of dry weight is in fact completely satisfactory also for this group of patients

Fat as percentage of dry weight compared with 24 hour total faecal fat own patients No attempt has been made to relate the faecal fat content (table IV) to the clinical conditions of the patients and due to their illness some of the patients may not have eaten any ordinary food in unlimited amount The table was compiled to make possible a comparison between the two ways of expressing faecal fat content.

seem reasonable to calculate also the total amount excreted daily. Random samples for several days may also be helpful due to the fluctuations in the individual which have been demonstrated in the previous article.

Conclusion

From the discussion it should be clear that it is satisfactory to express faecal fat content as percentage of dry weight when normal studies are made, and this way of expression may also be used as a guide in studying patients with idiopathic steatorrhoea and in most patients with unknown diagnoses (table IV), clinical observation of stools and defaecation being added to the chemical examination of faeces. Fig. 3 giving the 95 and 99 % confidence limits, could be used for easy judgement of the laboratory results. As a rule of thumb it can be remembered that between the ages 10—75 values above 20 % are almost always abnormal.

This simple way of examination with subjects on any ordinary food and using a random sample of faeces, which examination can easily be repeated, should greatly facilitate establishing the diagnosis of steatorrhoea. Only in borderline cases, e.g. when values are just outside the 9 % limits or very slightly abnormal, need faeces collections be made over a certain length of time to calculate the faecal fat in grams per day or balance tests be carried out to establish the coefficient of absorption.

Summary

Results expressed as percentage of dry weight from 133 determinations of faecal fat content in normals are presented. The individual excretion is analysed statistically. 10 samples from each of 10 healthy subjects showed large individual variation as well as large variations between individuals. When considering the effect of age and sex, it is found that a rise in total fat content is seen with increasing age, but that no difference can be seen related to sex. The best expression for fat percentage is thus found to be the equation fat-percentage = 0.1214 × age in years + 5.63.

Roughly it can be said that between the ages 10 and 75 the total fat percentage falls below 20 % in 95 % of healthy subjects eating an ordinary diet in unlimited amount. Often it should be lower (fig. 3). Comparisons are made with other studies on healthy subjects as well as on patients. It is concluded that only in borderline cases will various methods of expression slightly disagree. In such cases the clinical observation of the daily faecal amount may decide whether steatorrhoea exists or not, and the exact daily faecal fat content in grams may be calculated to help establishing the diagnosis.

Acknowledgement

The author acknowledges with gratitude the kindness of Arne Nielsen, Actuary, Institute of Human Genetics, University of Copenhagen, for taking the responsibility for the statistical evaluations of the normal material (tables I and II, figs. 2 and 3).

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Table IV (cont.)

Case no.	Age (yrs)	Faeces (g)	% Dry weight	Total fat % of dry weight	Total fat g	Comments
1959						
1,993	40	262	15.6	14.5	5.9	One-month interval
1,993	40	200	25.3	18.9	9.6	
2,169	39	655	11.6	16.4	12.5	
2,591	51	130	32.7	30 1	12.8	
2,806	59	80	33.8	15.0	4 1	
3,201	59	424	28.8	41.5	50.7	Identical with 190, but 4½ months later
3 697	26	34	31.9	18.6	2.0	
4,571	58	264	28.8	22.8	17.3	Three determinations on 3 consecutive days
4,571	58	217	21 6	20.3	9.5	
4,571	58	47	27.5	*0.8	2.7	

Percentage of fat low as compared with fat in grams.

Percentage of fat high as compared with fat in grams, 24-hour output of faeces very small (7,979 1 129 3,697 and 4 571) or rather small (3,247)

Results are shown as 48 analyses taken consecutively from the records at the laboratory of the Central Hospital Randers from patients where 24-hour collections had been made by request from the ward so that faecal nitrogen and faecal fat determinations could be made (table IV)

Eleven faecal fat determinations were normal 28 abnormal by the criteria of Cooke et al (6 g as the upper limit) all in agreement with my results Four of the remaining patients showed low percentages of fat as compared with fat in g (marked 1) two of those having extremely high percentages of dry weight (3055 and C 3842) for which reason the total must be high and two excreting great amounts of faeces (6785 and 149) The remaining 5 patients marked 2 had high percentages as compared to fat in grams, but in those cases the total amount of faeces was small for a 24-hour collection even though a sample of 50 g is enough for analytical purposes. In two of these five patients the collection was even less than 50 g Whether these samples really represented the entire daily excretion of the individual patients may be questioned. With a reasonable excretion of 100—110 g of faeces 4 of the values would be abnormal with both ways of expression (In cases 6183 and 7787 where 24-hour output of faeces is very small but other figures normal the amount of faeces might have trebled fat excretions in grams still remaining normal) As in the analyses in table III no complete uniformity is obtained When borderline values are found the examination should be repeated due to the variation in the individual and special attention should be paid in the ward to the exact collection of the stools and to the number of daily defaecations. This clinical observation in addition to the chemical determinations should enable one to decide whether the excretion is abnormal or not in those cases Of course it would then

seem reasonable to calculate also the total amount excreted daily. Random samples for several days may also be helpful due to the fluctuations in the individual which have been demonstrated in the previous article.

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Roughly it can be said that between the ages 10 and 75 the total fat percentage falls below 20 % in 95 of healthy subjects eating an ordinary diet in unlimited amount. Often it should be lower (fig. 3). Comparisons are made with other studies on healthy subjects as well as on patients. It is concluded that only in borderline cases will various methods of expression slightly disagree. In such cases the clinical observation of the daily faecal amount may decide whether steatorrhoea exists or not, and the exact daily faecal fat content in grams may be calculated to help establishing the diagnosis.

Acknowledgement

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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The Galactose Elimination Capacity in Control Subjects and in Patients with Cirrhosis of the Liver

By

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The elimination rate of galactose from the body is, theoretically, a valuable indicator of the liver function, particularly if the hepatic elimination capacity can be measured. The galactose elimination capacity may be determined with good approximation by an intravenous, single-injection galactose test (12). In order to study the clinical value of this test, it was applied to a group of control subjects and to patients with impaired liver function due to cirrhosis.

Methods

The patients were examined while they were still lying in their beds, 15 hours after [illegible] meal. Galactose was given intravenously, on the average 483 (S.D. 41) mg/kg body weight, dissolved in 100 ml of water and injected within 6 minutes. In the control subjects arterial samples were drawn at intervals of 3 min. during the period from 20 to 35 min. after the start of the injection, and then at intervals of 5 min. to 45 or 60 min. after the start. In patients with cirrhosis blood samples were drawn at intervals of 5 min. during the period from 20 to 60 or 75 min. after the start. The analytical procedure of Tygstrup et al. (11) was employed.

The rectilinear part of the plasma galactose concentration-time curve was delimited graphically, and the slope was calculated by regression. The galactose elimination capacity (GE) was calculated as

$$GE = (M - U) / (t_{c=0} + 7) \qquad (1)$$

where M is the amount injected, U the amount of galactose excreted in the urine, $t_{c=0}$ the extrapolated time when the concentration is zero, and 7 [illegible] correction for uneven distribution of galactose during the test (13).

Material

The control material consists of 96 in-patients in whom liver disease was not suspected. They suffered from a variety of disorders such as chronic bronchitis, asthma, constipation, heart disease without congestive failure, and (as the most frequent diagnosis) neurosis.

The material of patients with cirrhosis comprises 35 cases. In 32 of the patients the diagnosis was confirmed histologically (biopsy or autopsy). The etiology was probably alcoholism in 6 patients (1 female and 5 males), and viral hepatitis in 7 patients (4 females and 3 males). It was unknown in 22 patients (15 females and 7 males).

Submitted for publication August 12, 1963.

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Table I Mean values and SD of data from intravenous galactose tests in control subjects and patients with both the results of the initial test in each patient and the results of all the tests, including repeated tests in

	Age (yrs)		Body weight (kg)		BSA (sqm)		Galactose elimination capacity mg/min		Galactose elimination capacity mg/min/sqm		Slope of elimination curve (mg/l/min)	
Control subjects												
		Total	♀	○	♀	♂	♀	♂		Total	♀	○
No		96	32	64	32	64	32	64		96	32	64
Mean		35 7	60.3	67.3	1.63	1.81	438	482		267	47.6	42.3
SD		13 4	15 4	9 7	0.20	0.14	92	87		43	8.5	7.6
Var coeff (%)		37.5	25.5	14 1	12.5	7.9	21.0	18.1		16.0	17.9	18 9
Patients with cirrhosis												
	Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total
No.	35	101	35	101	35	101	35	101	35	101	33	101
Mean	50.6	55.0	63.0	61 4	1.64	1.65	235	244	142	149	17.9	20.7
SD	13.7	10.7	13.6	10.9	0.16	0.14	69	74	58	48	8.3	10.1
Var coeff. (%)	27 1	19 4	21.6	17.8	9.8	8.5	29.4	30.2	40.8	31.9	46.4	48.6

In 15 of the patients with cirrhosis the intravenous galactose test was repeated at different stages of the disease. Repeated tests were included only if the interval between them was at least 3 months, or if a significant change in the clinical state of the patient had occurred. In 5 patients more than 5 tests were performed (one patient 6 tests, two patients 8 tests, one patient 11 tests, and one patient 14 tests) Altogether 101 tests in patients with cirrhosis were included.

Results

Control subjects

In table I the mean values and the variation of the observations are given It appears that in some observations there is a significant difference between females and males. Thus the galactose elimination capacity is smaller in females This difference disappears, however if the elimination capacity is given in relation to body weight or to body surface area. By regression the following equations were found assuming linear relationships

$$GE = 171 + 4\ 54 \times kg \quad r = +0\ 607 \quad s = 72\ 6 \qquad (2)$$

and

$$GE = -45 + 293 \times sqm \quad r = +0\ 594 \quad s = 73\ 5 \qquad (3)$$

where GE is the galactose elimination capacity r is the correlation coefficient s is the standard deviation of GE from the regression line (residual standard deviation) In equation (2) the intercept is different from zero ($P < 0.001$) in equation (3) it is not significantly different. The partial correlation between galactose elimination and body weight for constant body surface is -0.170 and between galactose elimination and body surface for constant body weight $+0\ 074$ This indicates that body weight is the determining factor

In the control subjects no relation was found between the galactose elimination

cirrhosis. The control material is divided into females and males. In the patients with cirrhosis there are given same patients

Extrapolated time at c = 0 (min)		Residual concentration t 45 (mg/l)		Residual concentration t 60 (mg/l)		Excreted in urine (g)		Vol. of distribution (l)		Vol. of distribution % of body weight	
Control subjects											
	Total		Total		Total	♀	♂	♀	♂	♀	♂
	96		96		96	32	64	32	64	32	64
	60.6		456		200	3.03	3 69	10.5	13.0	17.8	19.5
	9 0		228		130	1 44	1.05	2.1	1.8	5.2	3.0
	14.9		50.0		65.0	47.6	28 4	19.9	14 8	17 7	15.6
Patients with cirrhosis											
Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total	Initial test	Total
35	101	34	101	35	101	35	101	35	101	35	101
115.9	115.9	1,047	1,044	849	797	2.74	2.93	15.8	14.5	25.2	23.8
34 9	40 2	250	257	310	305	1.66	1 47	4.1	3.7	4.7	4.7
30.1	34.7	24.7	24.6	36.5	38.3	60 6	50.2	25.9	25.6	18.7	19.7

capacity and the clinical diagnoses but the values tended to be lower in patients with peptic ulcer In the latter group consisting of 12 males, the galactose elimination capacity was on the average 440 mg/min S D 75) and when this is compared with the remaining mal controls (mean 489 mg min S D 89) the P-value of the difference is between 0 1 nd 0 05

The galactose limination capacity tended t decrease with increasing age, the decrease amounting t bout 1 mg min. per yea The P-value of the cor relation coefficient between elimination capacity per sq m and age is between 0 1 nd 0 05

The olume of distribution was smaller in the females than in the males, and this difference is not eliminated by relating the olume to body weight. I the female controls the relation, determined by regression was

$$V = 4.31 + 0.102 \times kg \quad r = +0.732 \tag{4}$$

and in the male controls

$$V = 7.67 + 0.079 \times kg \quad r = +0.397 \tag{5}$$

wher V is the volume of distribution the correlation coefficient. The regression coefficients of equation (4) and (5) are not significantly different ($0.4 > P > 0.2$) but the difference between the adjusted means, amounting to 1 88 l, is highly significant $P < 0.001$) The galactose elimination capacity was positively correlated to the volume of distribution (= + 0.318 $P < 0.001$)

Patients with cirrhosis

T ble I also shows the observations made in the patients with cirrhosis The mean values and standard deviations are given. There was no demonstrable dif ference between female and male patients as to galactose elimination capacity

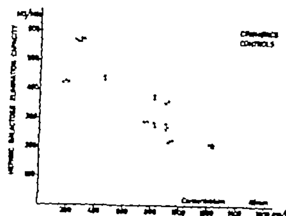


Fig 1 Correlation between galactose elimination capacity and the residual concentration of galactose 45 min. after the start of the injection

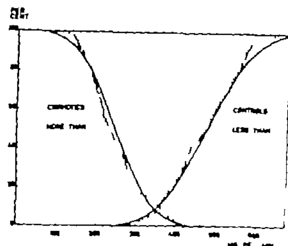


Fig 2 Distribution and overlapping of determinations of the galactose elimination capacity in control subjects and patients with cirrhosis. The lines show a normal distribution.

in the initial tests In both the initial and the total number of tests the galactose elimination capacity was not correlated to age, body weight or body surface area. The galactose elimination capacity was significantly higher ($P < 0.05$) in 39 tests made during prednisone treatment than in untreated patients (mean 263 mg/min S.D 73.7 and 232 mg/min S.D 71.8) In 40 observations made in patients with ascites or edema it was significantly lower ($P < 0.001$) than in patients without these signs (mean 210 mg/min S.D 60 and 267 mg/min., S.D 64)

The volume of distribution was significantly greater ($P < 0.001$) in patients with ascites or edema than in those without (mean 17.1 l, S.D 3.6, and 12.8 l S.D 2.7) The volume of distribution was smaller ($P < 0.02$) in the patients treated with prednisone than in the untreated group (mean 13.3 l S.D 3.4, and 15.2 l S.D 3.7) The volume of distribution was correlated to body weight as in the control material The regression equation was

$$V = 1.22 + 0.216 \times kg \quad r = +0.638 \qquad (6)$$

The intercept is not significantly different from zero and there was no significant difference between female and male patients. The galactose elimination capacity was negatively correlated to the volume of distribution ($r = -0.362$, $P < 0.001$)

The galactose elimination capacity and the residual concentrations 45 and 60 minutes after the injection are, as expected highly correlated (-0.773 and -0.805 respectively including both controls and cirrhotics) but still these concentrations are of limited value as a measure of the galactose elimination capacity particularly when the latter is high (fig 1) The standard deviation of the galactose elimination capacity from the regression line with the residual concentration at 45 minutes as independent variable, is 88 mg/min and with the concentration at 60 minutes as independent variable 82 mg/min.

It appears from table I that the urinary excretion of galactose is slightly but insignificantly smaller in the cirrhotics than in the controls. This is also the case when the excretion is expressed as a percentage

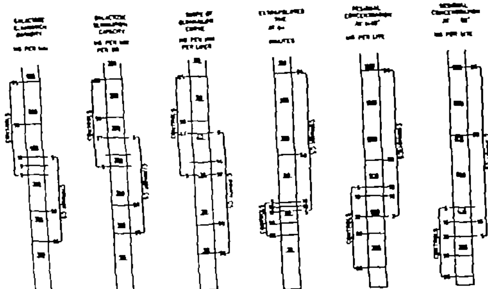


Fig. 3. Diagrammatic presentation of the distribution and overlapping of values in control subjects and patients with cirrhosis. Per cent of observations less than (upper part) or more than (lower part) the values indicated. The 5, 50, and 95 limits are indicated together with the percentages the over lap point

of the amount injected (11 0 and 10.0%, respectively) In agreement with this the correlation between galactose elimination capacity and amount excreted in the urine is positive in the total material (= + 0.196, P < 0.01)

The separation between the observations in patients with cirrhosis and those obtained in the control subjects cannot be calculated from the mean values and standard deviations given, because both groups are heterogenous and the values are not evenly distributed. The distribution and the overlapping of the values for the galactose elimination capacity of the controls and the cirrhotics may be seen from fig 2 The curve of normal distributions with the mean values and standard deviations given in table I are shown The theoretical curves show slightly better separation between controls and cirrhotics than the actual observations do In fig. 3 the overlapping of the values of the galactose elimination capacity and of some of the other data from the galactose test is depicted. The overlapping is smallest in the case of the galactose elimination capacity but it is of the same order of magnitude in all of them.

Discussion

Repeated determinations of the galactose elimination capacity in the same subject show that the reproducibility of the method is about 10 per cent (13) In the present control material the variation is about 20 per cent, and when related to body surface area, as estimated by the Du Bois height-weight chart, about 16 per cent. It seems more reasonable to correlate the elimination capacity with body surface area than with body weight

although the latter may be the decisive factor (cf the partial correlations) because the ratio elimination capacity/ body surface area was constant in the range examined and because other metabolic data e.g basal metabolic rate, *generally are considered to be related to* body surface area ("Rubner s law")

The significant correlation to body surface area may indicate that the elimination capacity depends on the size of the liver (liver mass L_m) If so the variation of the elimination capacity in relation to liver size in *normal* subjects should equal the reproducibility The greater variation of the elimination capacity per square meter body surface shows that either the correlation between *body surface and liver size or the cor* relation between liver size and elimination capacity is incomplete. The former correlation probably exists at least *inter species* (6) but it is not well defined in man *probably owing to the considerable* error involved in the determination of both parameters in relation to their absolute variation The latter correlation requires normal liver function and with the normality criteria used in the present work this may not be so in all the subjects For instance the tendency to lower values in the patients with peptic ulcer may reflect a slightly impaired liver function in this condition *(1)*

The influence of the age of the subjects on the elimination capacity has no practical significance but reduced liver function in old people has also been observed with other *tests (7) It may be* related to a diminution of liver size with age (10) or to a generally decreased metabolic activity The decrease in the galactose elimination capacity is of the same order of *magnitude* as *the* reduction in the basal metabolic rate (3) (about 0.25 % per year and about 0 4 % respectively)

The difference between female and male subjects may be due to differences in liver size and extracellular fluid volume. The extrapolated time at zero concentration the residual concentration at 45 minutes and that at 60 minutes were the same in females and males, probably because these observations are dependent on the amount injected which again was determined by the body weight. The difference in the amount ex *creted into the urine may probably* have the same explanation, since the ratio the amount excreted / the amount injected was identical in females and males.

The slope of the elimination curve in *the blood approximately equals the* ratio elimination capacity / volume of distribution It was higher in females than in males showing that in the females the volume was relatively more reduced than *the* elimination capacity *The volume of* distribution was about 18 of body weight in females and 20 % in males, i e. close to the values frequently given for the extracellular volume (2) The volume of distribution is not, however *a constant* fraction of the body weight the fraction decreases with increasing body weight (see equation (3) and (4)) In the extensive study of Moore et al (4) a *similar relationship between the* body weight and several parameters of body composition was found With regard to the extracellular volume, their regression equations are very similar to equation *(3) and (4) their regression* coefficients for females and males are the same and the difference between the adjusted means is statistically significant as with the present method

The volume of distribution expressed as a percentage of body weight was

higher in the cirrhotics than in the controls, and the ratio between body weight and volume of distribution was constant. Thus an increase in body weight results in greater retention of fluid in cirrhotics than in normal subjects — not unexpectedly since it is often due to an accumulation of ascites or edema. In patients with ascites the volume of distribution was on the average about 4 l higher than in non-ascitic cases. This is a minimum figure for the average amount of fluid retained, since concentration equilibration between ascites and plasma is not obtained during the test (unpublished observation). This introduces a systematic error in the determination of the hepatic galactose elimination capacity causing it t be over-estimated 13). The elimination capacity was significantly less in cases with ascites, however and the error is thus too small to hide the fact that the liver function usually is more reduced in patients with ascites. The same appears from the negative correlation between the elimination capacity and the volume of distribution in cirrhotics. In the control subjects the correlation was positive, probably because both the galactose elimination capacity and the volume of distribution were positively correlated with bod weight

Determination of the galactose elimination capacity is rather time-consuming If it could be assessed with reasonable accuracy from the concentration in single blood sample this would be a great practical advantage It ppears from fig 1 that the correlation between elimination capacity nd residual concentration is satisfactory only when the elimination capacity is low the retention test therefore is relatively insensitive. Zieve et al. (14) claim that the retention at 60 min. is a better measure of the liver function than that at 45 min. This is in agreement with the retention at 60 min being slightly better correlated with the galactose elimination capacity The urinary excretion of galactose after intravenous injection appears to be useless as an indicator of the elimination capacity This is in agreement with the previously observed reduction of the urinary clearance of galactose in patients with cirrhosis (12)

The degree of overlapping of the values in controls and cirrhotics is of interest as an indicator of the sensitivity of the test, i.e. its qualitative alue for deciding whether a patient has cirrhosis or not. If the aim is to minimize the total number of cases misclassified by the test, the overlap point must be used as the limit between normal and abnormal galactose elimination capacity provided that the material consists of the same number of cirrhotic and non-cirrhotic patients. If non-cirrhotic patients dominate, the limit should be fixed at a lower value, and vice versa The purpose of the test may be, however to exclude cirrhosis, in which case a higher limit should be used, e.g a limit exceeded by only 5 or 1 % of the patients with known cirrhosis. This is how the bromsulphalein-retention test is generally used, % retention being considered the highest normal limit. According to Zieve & Hill (15 16) 10 % of normal subjects and 9 8 of cirrhotic patients are misclassified if 8.2 retention is regarded as the dividing point but if 5 % retention is used, less than 5 % of cirrhotic patients nd about 30 of normals will be on the wrong side. Thus the test does not distinguish between cirrhotic and non-cirrhotic patients, but between patients who should be further tested and those who need not. From the data presented t seems unlikely that the

galactose elimination capacity will be any better than the bromsulphalein retention test for this kind of screening

When the present material is used to define the limit between cirrhotic and non-cirrhotic patients it should be borne in mind that the latter group does not consist of normal subjects in the physiological sense. Had this been the case, the separation would probably have been better but it would be less useful for the clinical purpose of the test. Yet it may be necessary to revise the normal range of the test if for example old patients, or patients with peptic ulcers are more frequent. One should also consider whether the material of cirrhotic patients is comparable with the present one. It is known that cirrhosis may vary clinically in different areas (9) moreover the number of mild cases may depend very much on the other diagnostic procedures used especially on the indications for liver biopsy In the present material there are rather too many mild cases, owing to the inclusion of repeated tests on some of the patients (only those surviving some time could undergo several tests)

The overlapping of the values found in controls and cirrhotics shown in fig 3 would probably have been smaller if the materials had been more homogenous. There has to be some overlapping however since in a liver biopsy cirrhotic changes do not preclude the presence of a normal amount of normally functioning liver cells Furthermore the wide range of the normal values means that in many subjects the elimination capacity may be reduced 20 % or more without falling below the normal limit. With a physiological variation of 20 (standard deviation) the minimum probable error of diagnosis in patients with e.g 50 loss of function will be 10 (8) A narrowing of the normal range (for instance, by relating it to the body surface area) is desirable, but as shown in fig 3 this serves to increase the overlapping presumably because the cirrhosis influences the determination of the body surface area A patient with marked wasting will have an elimination capacity per square meter that is disproportionately high, and when large amounts of ascites are present, determination of the body surface area loses its physiological meaning The separation could be improved only by relating the elimination capacity to the pre morbid body surface area, but this can rarely be done with accuracy

The conclusion is that the galactose elimination capacity just like the other known biochemical liver tests is not diagnostic for cirrhosis (5) The sensitivity of the test is utilized fully only if the subject can be made his own control as in the evaluation of different kinds of treatment or if groups of observations can be compared by statistical analysis.

Summary

The galactose elimination capacity was determined with the single injection technique in 96 control subjects. It was significantly smaller in females than in males, unless it was expressed in relation to body surface area

In 35 patients with cirrhosis of the liver 101 determinations were performed Nine per cent of the values in control subjects and patients with cirrhosis were overlapping and the separation could not be improved by relating the elimination capacity to body surface area The physiological variation implies that it may be impossible to decide if the liver function is normal or not from a single determination of the galactose elimina

tion capacity but if a reference value is obtainable either in the same subject or in a comparable group small variations in the liver function may be detectable.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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The Galactose Elimination Capacity in Relation to Clinical and Laboratory Findings in Patients with Cirrhosis

By

NIELS TYGSTRUP[1]

In the previous paper it was shown that the galactose elimination capacity determined by an intravenous, single-injection test, was generally lower in patients with cirrhosis of the liver than in control subjects, but the separation between them was incomplete (16). The aim of the present paper is to evaluate the galactose elimination capacity as a quantitative liver function test, by comparing it with other findings in patients with cirrhosis.

Methods and material

The following liver tests were performed by the methods indicated: galactose elimination capacity (17); serum electrophoresis (LKB equipment, veronal buffer pH 8.6, staining with amido-black B and evaluation for photometry); bromsulphalein retention (3, 10); prothrombin time (12); serum bilirubin (15) (using 3.32 as conversion factor); thymol turbidity (9) (extinction units, 1 cm light path.); oral galactose tolerance test (2, 11); serum alkaline phosphatase (6); serum glutamic-oxalacetic transaminase (8); serum glutamic-pyruvic transaminase (7). The tests were usually performed on the same day, but in some cases tests performed within the same week were included if the patient was in steady state clinically.

The results of the tests were divided into abnormality grades as shown in table I: Grade 0 is results within the normal limits. For the galactose elimination capacity the overlap point between controls and cirrhotics was regarded as the normal limit. The b-normal results were divided into 3 groups with approximately the same number of observations in each (with small deviations in order t void the separation of identical results). The abnormality score was calculated as the verage abnormality grade in five or six of the following tests: serum albumin, serum γ-globulin, bromsulphalein retention, prothrombin time, serum bilirubin, and thymol turbidity. The bnormality score was graded by dividing the scale into four equal parts.

The patient material is identical with that of the preceding paper. The observations were divided according to partly clinical, partly prognostic criteria.

On the basis of purely clinical assessment the status of the patients was evaluated as indicative of advanced liver failure, inter

Submitted for publication August 12, 1963.

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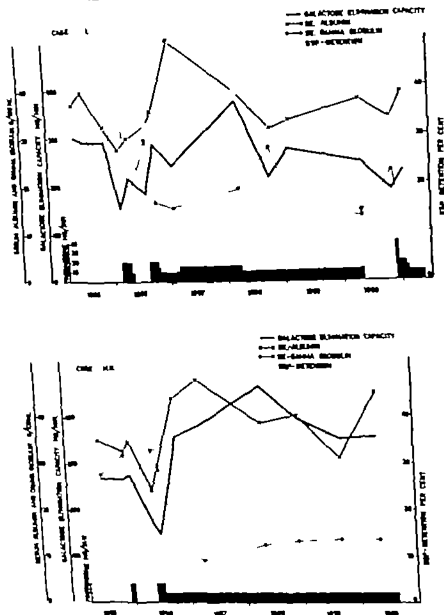


Fig. 1 Liver tests in two patients (nos. 4 and 5) with cirrhosis followed for relatively long periods.

lowing of barium showed esophageal varices. Liver biopsies in 1957 1958, and 1960 showed decreasing cellular infiltration and fibrosis.

Case A. K. Female, born 1905. Since 1956, increased BSR during autumn 1957 loss of weight and pronounced fatigue. Laparoscopy Dec. 1959, revealed small, nodular liver. Treated with prednisone from April 1959 to May 1960, and from July 1960 to Nov In March 1961 still very tired; liver function tests se table III Swallowing of

Table I Survey of the tests performed. Definitions of the abnormality grades and the abnormality score are given in Methods

	Mean	SD	No.	Range of the abnormality grades (% of observations)			
				0	1	2	3
Gal elimin. cap (mg/min)	244	74	101	≥ 357 (9)	< 357 – ≥ 250 (31)	< 250 – ≥ 200 (29)	< 200 (31)
Serum albumin (g/100 ml)	2.97	0.83	92	≥ 4.2 (7)	< 4.2 – ≥ 3.3 (28)	< 3.3 – ≥ 2.4 (32)	< 2.4 (32)
Serum γ-globulin (g/100 ml)	2.10	0.98	91	≤ 1.1 (11)	> 1.1 – ≤ 1.6 (29)	> 1.6 – ≤ 2.5 (30)	> 2.5 (29)
Bromsulphalein (% ret.)	28	14	86	≤ 5 (2)	> 5 – ≤ 20 (33)	> 20 – ≤ 35 (33)	> 35 (31)
Prothrombin (%)	67	25	82	≥ 85 (27)	< 85 – ≥ 63 (24)	< 63 – ≥ 47 (24)	< 47 (25)
Bilirubin (mg/100 ml)	1.9	2.1	99	≤ 1.0 (42)	> 1.0 – ≤ 1.5 (18)	> 1.5 – ≤ 2.5 (19)	> 2.5 (19)
Thymol turbid. (extinct. units)	0.33	0.22	95	≤ 0.13 (21)	> 0.13 – ≤ 0.28 (26)	> 0.28 – ≤ 0.48 (26)	> 0.48 (26)
Abnormal. score (mean grade)	1.6	0.7	96	≤ 0.8 (8)	> 0.8 – ≤ 1.5 (38)	> 1.5 – ≤ 2.3 (31)	> 2.3 (22)
Oral galactose (g in urine)	2.7	2.1	53	≤ 2.5 (57)	> 2.5 – ≤ 4.5 (15)	> 4.5 – ≤ 5.0 (15)	> 5.0 (13)
Alc. phosphatase (units/100 ml)	23.7	29.3	62	≤ 10 (37)	> 10 (63)		
GOT (units/ml)	3.7	3.6	39	≤ 1.7 (36)	> 1.7 (64)		
GPT (units/ml)	3.5	3.4	37	≤ 1.8 (46)	> 1.8 (54)		

mediate, or fully compensated. The first group comprises patients severely incapacitated owing to general physical debility or suffering from impending or manifest hepatic coma, not related to protein ingestion or gastrointestinal hemorrhage. The third group consists of a few patients who were fit and felt perfectly well, and the rest of the patients belonged to the second group.

Two groups were separated from the point of view of prognosis. One group consists of patients who died from progressive liver failure, less than 3 months after the test was performed (excluding patients who did not survive operations or hemorrhages occurring after the test). The other group comprises patients who were known to be alive one year after the test.

Case reports

Case 1 K. J. H. Female, born 1906. 1953, cholecystectomy. From 1953 to 1957 almost constantly slightly jaundiced, very tired, practically incapacitated. Since May 1957 treated with prednisone. In Nov. 1958 limited working capacity; liver function tests see table III. Intrasplenic pressure 22 mm Hg; splenoportography showed filling of esophageal varices. December hematemesis swal-

Table II. Correlation coefficients between liver tests in patients with cirrhosis

	Serum albumin	Serum γ-globulin	Bromsulphalein	Prothrombin	Bilirubin	Thymol turbid.	Abnormal score	Oral galactose	Alkaline phosphatase	GOT	GPT
Gal. elimin. cap.	0.616[1]	−0.450[1]	−0.519	0.483	−0.278	−0.318	−0.563[2]	−0.430	0.100	−0.180	0.097
Serum albumin	–	−0.320[1]	−0.466	0.507	−0.246	−0.139	−0.646[2]	−0.115	−0.083	−0.011	0.078
Serum γ-glob.	–	–	0.331	−0.411	0.335	0.680	0.727[2]	0.371	0.331	0.371	0.396
Bromsulphalein	–	–	–	−0.417[1]	0.373	0.321	0.695	0.079	0.047	0.317	0.231
Prothrombin	–	–	–	–	−0.427[1]	−0.247	−0.690[2]	−0.385	0.302	0.114	0.210
Bilirubin	–	–	–	–	–	0.327	0.636[2]	0.342	0.093	0.316	0.168
Thymol turbid.	–	–	–	–	–	–	0.646[2]	0.443	0.114	0.595	0.605
Abnormal score	–	–	–	–	–	–	–	0.396	0.115	0.428	0.189
Oral galactose	–	–	–	–	–	–	–	–	−0.244	−0.054	0.115
Alk. phosph. asc	–	–	–	–	–	–	–	–	–	0.550	0.419
GOT	–	–	–	–	–	–	–	–	–	–	0.639
Gal. conc. at 45′	−0.376	0.327	0.554[1]	−0.439	0.092	−0.058	0.241	0.310	−0.308	−0.150	−0.134
Gal. conc. at 60′	−0.568[1]	0.214	0.629[1]	−0.501	0.127	0.055	0.595[1]	0.356	−0.265	−0.025	−0.200

[1] Coefficients P < 0.001

[2] Coefficients between the abnormality score and tests from which the score was determined.

Table III. Liver tests in observations with major discrepancies between the abnormality grades of galactose elimination capacity and serum albumin

Case	Grade serum albumin minus grade gal. elimin. cap.	Gal. elimin. cap. (mg/min)	Serum albumin (g/100 ml)	Serum γ-globulin (g/100 ml)	BSP retention (% at 45′)	Prothrombin (%)	Serum bilirubin (mg/100 ml)	Thymol turbid. (extinct. units)
K. J. H.	−3	192	4.53	1.68	10	100	0.9	0.10
A. K.	−2	220	4.22	2.25	27	140	1.2	0.36
A. H.	−2	187	3.99	1.15	–	50	0.6	0.15
A. E. L. ( )	−2	240	3.03	1.51	30	88	2.2	0.70
A. E. L. (b)	−2	184	2.53	2.23	23	58	3.4	0.31
A. P. A.	+2	338	2.77	1.47	16	74	0.9	0.10
P. B. O.	+2	251	2.69	2.01	12	–	0.4	0.13
M. B. P.	+2	230	2.33	1.86	60	40	5.4	0.15

The correlation between some of the tests may be seen from their variations in two patients followed for longer periods during which major changes took place (fig. 1). Table II shows the correlation coefficients of the tests for the whole material. Fig. 2 depicts the relation between the abnormality grade of the

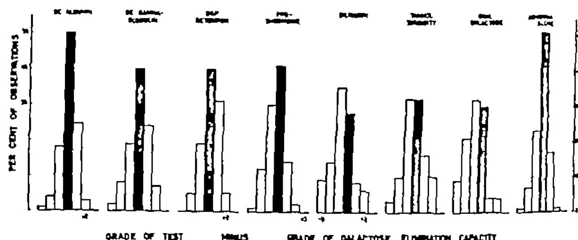


Fig 2 Difference between the abnormality grades of some liver tests and the galactose elimination capacity

barium esophageal varices. Liver biopsies 1958, 1960 and 1962 showed progressing cirrhotic changes.

Case 3 A. H. Female, born 1887 Ailing from 195. with right hypochondrial discomfort, fatigue and ankle edema. Cirrhosis diagnosed Nov 1956 Prednisone treatment from then to Aug 1958 when a large gastric ulcer was diagnosed. Liver biopsy Nov 1958 active portal cirrhosis. Nov 1959 precoma, lasting about two weeks. Feb 1961 relative well-being liver function tests see table III Laparoscopy small, coarsely nodular liver biopsy too little material intrasplenic pressure 14 mm Hg

Case 4 A. E. L. Female born 1898. From 1948, episodes of fever and jaundice from 1954 loss of weight and pronounced fatigue. Liver biopsy 1955 portal cirrhosis. Treatment with prednisone see fig 1 Feb 1957 still episodes as mentioned, but able to do her own housework liver function tests see table III (a) serum transaminases markedly elevated The clinical state was practically unchanged from 1957 to 1960 Prednisone treatment was discontinued on account of ankle edema, but started again 7 months later because of marked clinical deterioration liver function tests see table III (b) Intrasplenic pressure Aug 1960 was 33 mm Hg From Nov 1961 several small hematemeses.

Case 5. E. M. N Female, born 1904 From 1949 slight, recurrent jaundice, progressive fatigue. May 1955, constant right hypochondrial pain and subfebrile temperature. She was then totally incapacitated and bed-ridden. Liver biopsy active, post-necrotic cirrhosis. Temporary clinical improvement during short term prednisone treatment. Became able to do her own housework was afebrile and unjaundiced during continued prednisone therapy (see fig 1) From Aug 1959 relapse with brief episodes of right hypochondrial pain, fever and clay-colored stools, but no jaundice. Liver biopsies, Dec. 1958, May 1960, and April 1962 showed decreasing fibrosis and cellular infiltration. Intrasplenic pressure, May 1960, was 9 mm Hg swallowing of barium 1962 showed esophageal varices.

Results

Table I gives a survey of the liver tests and the limits of the abnormality grades. It appears that in some cases the standard deviations are close to or greater than, the mean values (e.g bilirubin and the serum enzymes) showing that the values are not normally distributed The same is indicated by the variation in the range of the abnormality grades The percentage of observations in grade 0 is an indication of the sensitivity of the tests.

Table II. Correlation coefficients between liver tests in patients with cirrhosis

	Serum albumin	Serum γ-globulin	Brom-sulphalein	Pro-thrombin	Bilirubin	Thymol turbid.	Abnormal score	Oral galactose	Alkaline phosphatase	GOT	GPT
Gal. elimin. cap.	0.616[1]	−0.430	−0.519	0.483[1]	−0.228	−0.318	−0.585	−0.430	0.100	−0.180	0.097
Serum albumin	—	−0.520	−0.466[1]	0.507[1]	−0.246	−0.139	−0.646	−0.113	−0.083	−0.011	0.078
Serum γ-glob.	—	—	0.331	−0.411[1]	0.335	0.690	0.727[2]	0.371	0.331	0.571	0.396
Bromsulphalein	—	—	—	−0.417	0.575	0.521	0.695	0.079	0.047	0.317	0.231
Prothrombin	—	—	—	—	−0.427	−0.247	−0.632	−0.385	0.302	0.114	0.210
Bilirubin	—	—	—	—	—	0.327	0.636[2]	0.342	0.093	0.516	0.198
Thymol turbid.	—	—	—	—	—	—	0.646	0.445	0.114	[illegible]	0.605
Abnormal score	—	—	—	—	—	—	—	0.396	0.115	0.428	0.169
Oral galactose	—	—	—	—	—	—	—	—	−0.244	−0.054	0.115
Alk. phosph. ase	—	—	—	—	—	—	—	—	—	0.550[1]	0.419
GOT	—	—	—	—	—	—	—	—	—	—	0.639
Gal. conc. at 45	−0.376	0.327	0.334[1]	−0.438	0.022	−0.098	0.241	0.310	−0.308	−0.150	−0.154
Gal. conc. at 60′	−0.568	0.214	0.629[1]	−0.501	0.127	0.053	0.395	0.358	−0.265	−0.023	−0.200

[1] Coefficients $P < 0.001$

[2] Coefficients between the abnormality score and tests from which the score was determined.

Table III. Liver tests in observations with major discrepancies between the abnormality grades of galactose elimination capacity and serum albumin

Case	Grade serum albumin minus grade gal. elimin. cap.	Gal. elimin. cap. (mg/min)	Serum albumin (g/100 ml)	Serum γ-globulin (g/100 ml)	BSP retention (% at 45′)	Pro-thrombin (%)	Serum bilirubin (mg/100 ml)	Thymol turbid. (extinct. units)
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N. K.	−2	220	4.22	2.23	27	140	1.2	0.36
A. H	2	107	3.89	1.15	—	50	0.6	0.15
A. F. L. (a)	−2	240	3.03	1.51	30	88	2.2	0.70
A. E. L. (b)	−2	184	3.33	2.23	23	58	3.4	0.31
N. P. A	+2	158	2.77	1.47	16	74	0.9	0.10
F. B. O	+2	251	2.89	1.01	1	—	0.4	0.13
M. S. P	−2	250	2.33	1.88	60	40	9.4	0.15

The correlation between some of the tests may be seen from their variations in two patients followed for longer periods during which major changes took place (fig. 1). Table II shows the correlation coefficients of the tests for the whole material. Fig. 2 depicts the relation between the abnormality grade of the

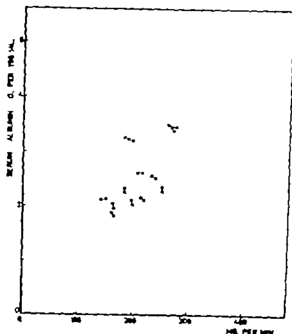


Fig 3 Correlation between the galactose elimination capacity and serum albumin.

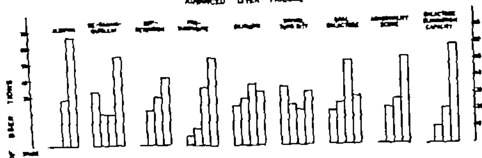
galactose elimination capacity and that of some other tests Both in table II and in fig 2 the galactose elimination capacity is best correlated with serum albumin and the abnormality score, and slightly less well with bromsulphalein, γ-globulin and prothrombin.

As the correlation between galactose elimination capacity and serum albumin is of particular interest, major discrepancies were examined more closely It was considered a major discrepancy if the difference between the abnormality grades was greater than one. This was found in 8 cases, shown in table III In the last 3 cases (A.P.A. F B O and M.S.P) the galactose elimination capacity was less abnormal than serum albumin but it was so close to the border between the grades that a change of less than one per cent might reduce the difference in abnormality grades to one, so the discrepancy may not be real. The same applies to the second case (A K.) in which a comparable change in serum albumin might have the same effect. In the rest of the eight cases the serum albumin is definitely less abnormal than the galactose elimination capacity and those cases are easily recognized in fig 3, showing the correlation between galactose elimination capacity and serum albumin in the whole material

In table IV are given the mean values of the tests in the clinical and prognostic groups and the t values of the difference between the means are given as a measure of the separation of the groups. Fig 4 shows how frequently observations in the different abnormality grades were found in two of the groups, in relation to their frequency in the total material (table I) Fig 4 thus illustrates how the grouping is predicted from the tests.

Discussion

The physiological and pathological significance of the common liver tests is not completely understood, and no single test is diagnostic of cirrhosis. The different tests are intended to quantify different aspects of the cirrhotic process e.g the impairment of the metabolic and the excretory function and the degree of inflammation and necrosis in the liver Each of these aspects consists of numerous, mostly unknown factors and other aspects, such as circulatory disarrangement may be equally important Fig 5 shows a hypothetical relation between the tests studied in the present paper and the three factors just mentioned The lines between the tests represent the most significant correlations (i e $P < 0.001$) given in table II The three factors are not completely separated in the figure as they must to some extent be dependent on each other and certain tests are



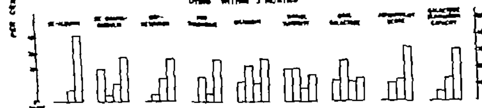


Fig. 4. Abnormality grades of liver tests in clinical and prognostic group of patients. The number of observations of each grade is expressed as percentage of all observations of that grade.

Table IV. Mean values in the clinical and prognostic groups and t-values of the difference between the group

Test	Clinical groups					Prognostic groups		
	A Mean	Diff./SE of diff. (A−B)	B Mean	Diff./SE of diff (B−C)	C Mean	D Mean	Diff./SE of diff. (D−E)	E Mean
Gal. elimin. cap.	190	5.07	258	4.34	381	182	4.50	269
Serum albumin	2.24	6.41	3.23	3.71	4.50	2.14	*6.16	3.39
Serum γ-globulin	2.42	1.67	2.05	*2.72	0.82	2.57	2.29	1.93
Bromsulphalein	33	1.73	27	*2.83	8	34	3.09	22
Prothrombin	50	3.40	71	0.92	83	0	2.63	74
Bilirubin	2.0	0.04	2.0	1.12	0.7	2.1	0.79	1.7
Thymol turbid.	0.34	0.50	0.35	2.47	0.08	0.52	0.55	0.54
Abnormal score	1.9	2.42	1.6	3.21	0.6	2.1	3.37	1.4
Oral galactose	3.2	0.67	2.7	1.40	1.2	3.1	0.37	2.8
Alkaline phosphatase	18.8	0.81	26.0	0.80	8.4	22.3	0.52	25.4
GOT	4.0	0.11	3.8	0.73	2.5	6.9	1.79	3.7
GPT	2.7	0.58	3.6	0.21	4.0	2.6	1.17	3.8
Gal. conc. 45′	1,198	3.80	956	1.92	775	1,178	3.36	948
Gal. conc. 60′	1,030	4.09	724	*2.88	500	1,012	*4.38	651

A advanced liver failure (N = 29) B intermediate (N = 67) C fully compensated, (N = 5); D dying within 3 months, (N = 18) E alive one year later (N = 38) (N = number of determinations of galactose elimination capacity)

* P < 0.001

* P < 0.05.

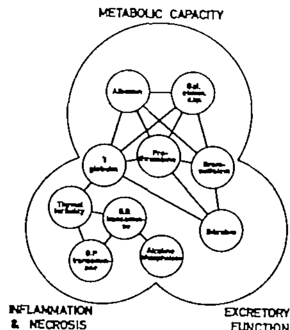


Fig 5 Hypothetical relation between three aspects of liver function in cirrhosis and liver tests. Lines between symbols of tests indicate that the correlation between them in the present material was highly significant ($P < 0.001$)

placed at the borders between them. Thus bromsulphalein depends not only on the excretory function but also on the metabolic capacity as shown by the high correlation to albumin and galactose elimination capacity Similarly γ-globulin and albumin may be interdependent through factors controlling the protein metabolism. The partial correlation between galactose elimination and γ-globulin for constant albumin, was not significant ($r = 0.164$ $P > 0.1$) The correlation between galactose elimination and the four tests (albumin, γ-globulin prothrombin, and bromsulphalein) in the rest of the cases remained significant — although at a lower level — when one of the others was kept constant. So the correlation between galactose elimination and γ-globulin is not genuine and γ globulin probably does not really belong to the group of tests indicating the metabolic activity Alkaline phosphatas is generally regarded as a test of the ex cretory function in liver disease, but in the present material it is better correlated with the transaminases, assumed to be indicators of destruction of cells (19). Probably serum alkaline phosphatase under certain conditions may also come from the hepatic cells (15) explaining a correlation with transaminases.

Deductions from correlations in a material of this kind are bound to be limited. Some degree of correlation may be due to a tendency to equal impair ment of independent functions in different stages of cirrhosis. Tests of the same function may also show a rather low correlation if their distribution is different especially if one test is much more sensitive than the other

It is reasonable to assume, however that the highest degrees of correlation reflect similarities in the mechanisms of the tests provided they are methodologically independent. The correlation between serum albumin and the galactose elimination capacity is the highest found among methodologically independent tests in the present material. Presumably this is because both depend on the metabolic capacity of the liver This dependence is apparently not the same in the two tests, since the relation between albumin and galactose elimination varies considerably (fig 3) Serum albumin is generally recognized to be a reliable quantitative liver test in cirrhosis (14) and it is significantly correlated with histological liver cell damage (13) but its relation to the hepatic metabolic capacity must be via the rate of albumin synthesis (16) which is only one of the factors determining the serum albumin concentration The galactose elimination may likewise depend on other factors such

as extrahepatic removal, the actual NADH/NAD ratio in the liver (4) or specific enzyme defects (1) The analysis of the observations in which albumin and galactose elimination capacity gave definitely divergent results does not reveal which test is the better measure of the hepatic metabolic capacity In all the cases the cirrhosis was clinically important, but probably in a quiescent or remittent phase neither of the tests can be deemed definitely misleading

From the practical point of view the conclusion may be that one of two highly correlated tests is superfluous. Thus Zieve and Hill (20) have shown that a certain combination of the bromsulphalein, zinc sulphate hippuric acid, and urine coproporphyrin tests permitted good separation of controls and cirrhotics, and nothing was gained by the inclusion of bilirubin, thymol turbidity per cent cholesterol esters, galactose tolerance (retention at 60 min.) and urine urobilinogen, although several of the latter tests, particularly galactose tolerance, had a considerable discriminative effectiveness of their own.

For laboratory evaluation of patients with known cirrhosis this conclusion is probably not valid. Statistically serum albumin was clearly the best among the tests examined (fig 4) although the clinical classification was very rough. This does not imply however that serum albumin is invariably reliable Serum albumin is relatively insensitive test for the detection of slight or transient liver injury and it is frequently reduced in conditions without liver disease. Nor are other tests superfluous even the transaminases, which show very low discriminative effectiveness (table IV) are of indisputable value in certain cases. One transaminase will probably suffice, however and the G O transaminase seems to be preferable. The abnormality score is not better than the best of its components, i.e. serum albumin the same was found regarding the ratio albumin/γ-globulin a fixed combination of several tests is hardly advantageous.

With our present knowledge the interpretation of liver tests is mainly based on clinical experience of a limited number of tests, and on a certain amount of imagination Much more must be known about the relevant liver functions and their measurement before a rational battery of liver tests can be proposed. The results of the present paper suggest that the metabolic capacity is an important factor in cirrhosis, and that the galactose elimination capacity is a measure of this factor In many cases a determination of the serum albumin will give the same information, but as this is a rather non-specific test, determination of the galactose elimination capacity may be desirable.

Summary

The results of 101 determinations of the galactose elimination capacity in patients with cirrhosis are compared with the results of other liver tests. A high degree of correlation was found with tests assumed to depend on the metabolic capacity of the liver and in particular with the serum albumin concentration. Grouping of the material according to clinical and prognostic criteria showed that serum albumin gave the best, and galactose elimination capacity the next best, agreement with these criteria. It is concluded that the metabolic capacity is an important factor in cirrhosis, and that the galactose elimination capacity is a measure of this factor

Acknowledgements

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My thanks are due to Mrs. Lis Hansen for skillful technical assistance, and to Dr K. Winkler M.D for invaluable help during all phases of the work. Support was received from the Danish State Research Foundation.

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Acta Medica Scandinavica, Vol. 175, fasc. 3, 1964

From Rigshospitalet, Medical Department B (Former chief E. Warburg, M. D present chief. A. Tybjerg Hansen, M. D) Radiological Department (Chief. Gregers Thomsen, M. D) and Isotope Laboratory (In charge: T Munkner M. D.) University Hospital of Copenhagen, Denmark

Body Composition in Heart Disease

Total Exchangeable Potassium, Total Exchangeable Sodium, Total Exchangeable Chlorid and Derived Values for Body Composition in Cardiac Disease with and without Edema

By

Knud H. Olesen

Clinical observations have long recognized that subjects with cardiac edema may present signs of wasting of muscle tissue and body fat (29 30, 31 38, 79) While several aspects of body composition in edematous heart failure have been clarified through metabolic balance studies (5, 20, 22 33 34 35 43 47) a simultaneous measurement of tissue wasting and extracellular edema has first become accessible with the application of multiple isotope dilution methods to the study of body composition in man (45 49 50, 53)

Multiple tracer studies in subjects with cardiac edema have indicated changes in composition characterized by a decrease of total exchangeable potassium, body cell mass and body fat and an increase of total exchangeable sodium, total exchangeable chloride and extracellular water when these values are expressed on a body weight basis (7 52 53 59) Consistent with these findings dilution studies with one or two isotopes have tended to show that cardiac edema was associated with a decreased total exchangeable potassium (3 26 51 55) and an increased total exchangeable chloride (9 18, 24 65) and increased total exchangeabl sodium (1 2, 17 26 28 51 64 73 76, 80 81 85)

An extension of isotope dilution studies in cardiac edema is desirable, however because of the limited number of patients studied with multipl tracer techniques and because of the difficulties involved in measuring and interpreting changes in composition in edematous patients. The rates of equilibration of isotopes may be delayed in edematous patients (54 62) and variations in exchangeability of el-

Submitted for publication August 12, 1963.

Acknowledgements

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Body Composition in Heart Disease

Total Exchangeable Potassium, Total Exchangeable Sodium, Total Exchangeable Chloride and Derived Values for Body Composition in Cardiac Disease with and without Edema

By

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Clinical observations have long recognized that subjects with cardiac edema may present signs of wasting of muscle tissue and body fat (29 30 31 58, 79). While several aspects of body composition in edematous heart failure have been clarified through metabolic balance studies (5 20, 22, 33 34 35 43 47) a simultaneous measurement of tissue wasting and extracellular edema has first become accessible with the application of multiple isotope dilution methods to the study of body composition in man (43 49 50 53).

Multiple tracer studies in subjects with cardiac edema have indicated changes in composition characterized by a decrease of total exchangeable potassium, body cell mass and body fat and an increase of total exchangeable sodium, total exchangeable chloride and extracellular water when these values are expressed on a body weight basis (7 52 53 59). Consistent with these findings dilution studies with one or two isotopes have tended to show that cardiac edema was associated with a decreased total exchangeable potassium (3, 26 51 55) and an increased total exchangeable chloride (9 18 24 65) and increased total exchangeable sodium (1 2, 17 26 28, 51 64 75 76 80 81 85).

An extension of isotope dilution studies in cardiac edema is desirable, however because of the limited number of patients studied with multiple tracer techniques and because of the difficulties involved in measuring and interpreting changes in composition in edematous patients. The rates of equilibration of isotopes may be delayed in edematous patients (54 62) and variations in exchangeability of el-

Submitted for publication August 12, 1963.

ectrolytes with isotopes must be considered (4 51 85) The variations in body composition with sex (19 41 53 66) age (19 42 53 66) and body weight (41 42 53 66) make it necessary to ensure that changes ascribed to a disease are not a reflection of these other factors. The discrepancy between reports indicating a retention of sodium in excess of the gain of extracellular water in cardiac disease (10 21 76) and studies failing to confirm this general trend (19 53) points up the need for additional studies.

The objectives of the present investigation were 1) to compare total exchangeable potassium (K_e) total exchangeable sodium (Na_e) 24 Na space, total exchangeable chloride (Cl_e) 82 Br space and derived values for body composition in edematous and nonedematous groups of cardiac patients of similar sex age and body weight with particular reference to the tissue wasting aspects and to the distribution of sodium 2) to analyse the patterns of composition in edematous and nonedematous heart disease on the background of normal body composition predicted from body weight and 3) to discuss limitations of the data presented In preliminary studies the rates of equilibration of isotopes were examined and variations in exchangeability of body electrolytes with isotopes were appraised by comparison of changes in body contents measured by sequential tracer studies and by metabolic balance methods.

Potassium 42 24 Na and 82 Br were used as tracers, and the results were expressed as total quantities. A comparison of edematous and nonedematous cardiac patients of similar age, sex and body weight revealed that cardiac edema was associated with a decrease of total exchangeable potassium, body cell mass and probably body fat, and an absolute increase of total exchangeable sodium, 24 Na space total exchangeable chloride, 82 Br space, total extracellular water total extracellular sodium and Na_e K_e ratio The increase of total exchangeable sodium could be accounted for by increment of extracellular sodium alone. A comparison of the pattern of body composition in edematous subjects and predicted normal body composition confirmed the findings outlined above, whereas the body composition of nonedematous cardiac patients differed little from predicted normal values, except for a rise of Na_e K_e ratio.

Evidence is presented that the results obtained are not due to differences in rates of equilibration of isotopes or to variations in exchangeability of body electrolytes with isotopes.

Material

Forty-eight patients with an unequivocal diagnosis of heart disease were studied. They were divided into the following groups for purposes of comparison 1) twelve edematous males, 2) twelve nonedematous males, 3) twelve edematous females, and 4) twelve nonedematous females. Within either sex the edematous and nonedematous groups have approximately the same average age and the same mean body weight and body height.

The series includes patients with rheumatic, arteriosclerotic and congenital heart disease and a few cases of myocarditis and constrictive pericarditis as listed in table I All patients received the customary treatment for their disease, as shown in the table, up to the week of study When benzothiadiazines were administered a supplement of potassium chloride (40 mEq K daily) was always given, except when the potassium-saving spironolactones were used. In the nonedematous group two thirds of the patients received digitalis, and about half had been given diuretics. In the edematous group nearly all patients had earlier been treated with diuretics.

Table I Clinical and laboratory data

Case no.	Diagnosis	Treatment	Age (yrs)	Body weight (kg)	Body height (cm)	Serum Na (mEq/l)	Serum Cl (mEq/l)	Serum K (mEq/l)	24 Na space (l)	82 Br space (l)
Edematous heart disease										
Males										
1	ASHD	D, B, T	48	63.0	166	143	96	4.0	34.0	33.0
2	Myocarditis	D B	23	65.0	175	140	101	4.4	23.2	27.0
3	RHD AI	D B, T	64	75.8	173	141	97	3.7	41.1	42.7
4	RHD, MI, MS	D B	50	68.0	177	137	96	4.4	29.1	27.1
5	CP	D, B, S	28	67.0	170	140	100	3.8	31.9	35.4
6	CHD, AC	D, B	48	68.3	169	137	100	3.7	33.1	31.4
7	ASHD	D	62	56.5	173	143	96	4.4	22.4	21.6
8	RHD, MI	D, B, S	56	71.8	173	131	95	3.2	31.4	31.7
9	RHD, MI, MS	D, B, S	42	60.0	170	134	91	4.3	23.1	23.9
10	Myocarditis	D, B	39	73.5	178	134	87	4.0	35.0	36.6
11	CP	D,B,S,T	40	88.4	184	128	90	4.3	49.8	54.2
12	RHD MS	D, B, S	40	63.5	178	139	95	4.3	23.1	22.9
Nonedematous heart disease										
Males										
13	RHD MI	D B	50	71.5	177	143	95	4.0	24.5	24.0
14	RHD, MS	D	39	62.4	178	141	95	4.6	21.8	22.1
15	ASHD	D, B	48	54.3	166	143	94	4.2	20.4	18.1
16	CP	D, B, S	28	59.5	170	133	86	5.0	18.3	19.8
17	CHD, ASD	None	62	73.0	164	148	95	4.8	18.5	19.0
18	ASHD	D, B, S	31	54.8	174	135	92	4.3	20.7	20.1
19	CP	D	41	85.6	184	141	100	4.4	30.5	29.5
20	RHD MS	None	42	75.3	176	143	103	4.7	24.5	25.7
21	RHD AS	None	29	75.9	180	145	102	4.6	23.9	23.7
22	RHD, MI	D B	43	71.5	167	141	104	4.5	20.8	20.0
23	CHD AC	None	46	63.0	167	145	103	4.2	19.9	18.4
24	CHD, ASD	D	32	68.3	180	144	104	4.2	23.0	22.6
Edematous heart disease										
Females										
25	RHD, MI	D, B, S	46	50.3	162	139	95	4.1	26.6	26.9
26	ASHD	B	58	57.5	163	143	96	4.6	22.5	21.8
27	RHD, MI, MS	D, B, S	49	60.3	162	139	96	4.1	26.9	27.5
28	RHD, MS, MI	D, B	50	53.3	168	144	104	4.9	22.7	22.0
29	ASHD	D, B, S	54	76.3	170	142	93	3.4	40.0	42.2
30	Myocarditis	D B	24	49.2	157	141	98	4.1	24.6	23.9
31	RHD, MS	D, B, S, T	57	46.3	158	138	99	4.6	17.8	17.6
32	RHD, MS, AI	D B, S	53	62.2	170	145	96	4.8	28.6	30.4
33	RHD MS	D, B	54	57.3	157	138	94	3.9	21.1	20.1
34	RHD, MS	D, B	56	46.5	156	140	85	4.4	20.7	20.4
35	RHD, MI	D B	57	49.7	151	141	101	4.4	18.5	17.5
36	RHD, MI, MS	D B T	50	57.7	174	128	79	5.0	27.0	29.0

Table I (cont)

Case no.	Diagnosis	Treatment	Age (yrs)	Body weight (kg)	Body height (cm)	Serum Na (mEq /l)	Serum Cl (mEq /l)	Serum K (mEq /l)	24 Na space (l)	82 Br space (l)
Nonedematous heart disease										
Females										
37	CHD PD	None	46	58.0	160	139	104	4.2	19.4	19.1
38	RHD MI	D B S	58	38.0	151	138	101	4.4	12.8	13.2
39	RHD MS	D	44	44.5	139	145	99	4 1	13.5	14.1
40	RHD MS	D	46	59.3	160	142	105	4.2	18.0	16.5
41	RHD MS	None	39	54 1	163	143	103	4.3	17.3	18.1
4	ASHD	D B, S	68	53.3	152	147	101	4.5	14.0	13.9
43	ASHD	D B, S	51	58.0	170	133	91	5.5	19.9	21.1
44	RHD MS	D B, S	57	45.6	159	138	97	3.1	14.7	14.5
45	ASHD	D B	58	46.7	163	141	84	3.7	14.3	14.1
46	CHD, ASD	D	48	57.8	152	138	98	4.4	17.8	17.1
47	RHD MS AI	D	36	53.8	170	142	103	4.0	18.9	18.2
48	RHD MS	None	42	54.5	156	143	104	4 4	16.9	17.3

ASHD arteriosclerotic heart disease RHD rheumatic heart disease CHD: congenital heart disease AI aortic incompetence MI mitral incompetence MS mitral stenosis CP constrictive pericarditis, AC aortic coarctation ASD atrial septal defect AS aortic stenosis PD patent duct.

Treatment D digitalis B benzothiadiazines S spironolactones T thiomerin.

Methods

Total exchangeable potassium, total exchangeable sodium, 24 Na space total exchangeable chloride and 82 Br space were measured according to a modification of the method described by Veall and Vetter (77) The isotopes used were 42 K 24 Na and 82 Br delivered from the Radiochemical Centre Amersham, England, or from the Danish Atomic Centre Risø Denmark

The procedure was as follows 50 to 100 μc 42 K, 50 to 100 μc 24 Na, and 15 μc 82 Br were administered orally in saline at 8 a. m. The urine was collected overnight allowing a correction for urinary loss of isotopes during the period of equilibration of 24 hours. Next morning the patients were kept fasting After a complete voiding two consecutive spot urines for assay of 42 K were collected over a three hours period venous samples for assay of 24 Na and 82 Br were taken 24 and 25 hours after administration of isotopes, and the body weight was measured.

The assay of 24 Na and 82 Br was based upon analysis of plasma samples. From each venipuncture duplicate samples, and diluted standards of 24 Na and 82 Br were subjected to gamma scintillation counting in a well scintillation detector (Na J(Tl) 1 7/8 × 2 inches) connected with a two-channel pulse height analyser with joined scalers (Tracerlab) on channel adjusted to optimal sensitivity for 24 Na, the other adjusted for 82 Br The urinary losses of 82 Br were counted on a 24-hour-sample pretreated with Amberlite resin IR 120, removing the cations with a measured efficiency of 99 97 Similarly the losses of 24 Na were measured on urine samples treated with Amberlite resin IRA 400, removing the anions with a measured efficiency of 99.8 %

The assay for 42 K was based upon analysis of spot urines. After removal of 82 Br with Amberlite resin IRA 400 duplicates of the spot urines, and diluted standards of 42 K and 24

Na were subjected to counting in a thick walled liquid beta tube G-M counter type M 6 (Twentieth Century Electronics, Surrey England) connected with a scaler (Tracerlab) and to gamma counting in a well scintillation detector connected with a pulse height analyzer with joined scaler adjusted to optimal sensitivity for 24 Na. The urinary losses of 42 K and 24 Na were determined in similar manner

Plasma sodium and potassium were measured by flame photometry plasma chloride by potentiometric titration. The potassium concentration of spot urines was measured by flame photometry using K standards with N contents approximating the sodium concentration of the spot urine examined.

In a smaller group of edematous patients the rates of equilibration of isotopes were studied by comparison of the specific activities of tracers in samples taken after 24 36 and 48 hours. The alterations of body sodium and potassium in sequential isotope studies with intervals of one to two weeks were compared to the results obtained in simultaneous metabolic balance studies in order to test the possibility of variations in exchangeability of electrolytes with isotopes.

Calculations

The count rates obtained from plasma samples represent the sum of the contributions given by 24 Na and 82 Br since the effect of 42 K under the conditions of measurement is negligeable.

The count rate due to 24 Na in the Na channel (S_{Na}) is derived from the formula

$$S_{Na} = \frac{N_{Na} - n\,N_{Br}}{1 - m\,n}$$

where

N_{Na} = Count rate of plasma sample in Na channel

N_{Br} = Count rate of plasma sample in Br channel

$$n = \frac{\text{Count rate of 82 Br standard in Na channel}}{\text{Count rate of 82 Br standard in Br channel}}$$

$$m = \frac{\text{Count rate of 24 Na standard in Br channel}}{\text{Count rate of 24 Na standard in Na channel}}$$

Similarly the count rates due to 82 Br in the Br channel (S_{Br}) may be calculated from the formula

$$S_{Br} = \frac{N_{Br} - m\,N_{Na}}{1 - m\,n}$$

$$\text{or } S_{Br} = N_{Br} - m\,S_{Na}$$

After correction for time decay the 24 Na space and the 82 Br space may be calculated

$$\text{24 Na space} = \frac{\text{24 Na counts administered} - \text{24 Na counts excreted}}{S_{Na}/l}$$

$$\text{82 Br space} = \frac{\text{82 Br counts administered} - \text{82 Br counts excreted}}{S_{Br}/l}$$

These spaces are the theoretical volumes of dilution of these isotopes if they were distributed throughout in the same concentrational relationships as in plasma. From the spaces the total exchangeable electrolytes are determined according to the formulas

Na_e = (24 Na space) (serum sodium concentration)

Cl_e = (82 Br space) (serum chloride concentration)

The total exchangeable potassium is determined from the beta and gamma counting of spot urines according to the method described by Veall and Vetter (77)

The average urinary losses of isotopes during the period of equilibration, expressed as a percentage of the doses administered were For 42 K, 4.2 %, for 24 Na, 3.2 %, and for 82 Br 3 1 %. No significant difference was found between edematous and nonedematous groups.

Errors of the method

The coefficients of variation of duplicate determinations of total exchangeable electro-

lytes and of volumes of dilution in cardiac subjects were For 24 Na space 2.7 % for Na_e, 3.0 % for 82 Br space, 2.8 % for Cl_e, 3 0 and for K_e 4 8 The coefficients of variation of reproducibility with an interval of one or two weeks in normals and in stabilized cardiacs were For Na_e, 3 5 % $(n = 10)$ for Cl_e, 3.8 $(n = 10)$ and for K_e, 4.3 % $(n = 10)$

Derived values

From the direct measurements of K_e, Na_e, 24 Na space Cl_e, 82 Br space and body weight (B Wt.) the following deductions are made

a. Body cell mass (BCM)

The total body cell mass, "the body engine" comprises cellular solids and water and is calculated from the formula

$$BCM = (K_e)\ 8.33.$$

This formula is based upon the fact that potassium is essentially an intracellular ion and assumes a normal composition of the cell mass (53) and it should be noted that it may lead to a severe underestimation of the true cell mass if a marked potassium loss from cells has occurred

b. Extracellular water (ECW)

In this context ECW indicates the total extracellular water of plasma, interstitial fluid, lymph, dense connective tissue, cartilage, bone and the transcellular fluids (synovial fluid, and gastrointestinal water) The extracellular water is derived from the formula

$$ECW = 0.86\ (82\ Br\ space)$$

This formula is derived from the data presented by Moore et al. (53) These authors measured 82 Br space plasma volume (PV). and red cell volume (RCV) and derived ECW from the formula

$$ECW = \frac{Br\ space - PV - 0.6\ RCV}{\left(\frac{1.02}{0.92}\right)} + 0.92\ PV$$

which includes a correction for red cell chloride (bromide) and for the difference in chloride (bromide) concentration of plasma and interstitial fluid by help of the factors 0.92 (correcting for plasma solids) and 1 02 (correcting for the Donnan effect across capillary membranes)

A correction of Br space with a constant of 0 86 was chosen for the present series, since this factor in normal individuals, and in nonedematous and edematous cardiac subjects in both sexes predicted the same average values for ECW as obtained by the more complicated formula presented above

c. Extracellular solids and body fat (ECS + fat)

The sum of extracellular solids, including the total noncellular solids of the skeleton, and total body fat may be derived from the formula

$$ECS + fat = B.\ Wt. - BCM - ECW$$

d. Total extracellular sodium (ECNa)

Total extracellular sodium is calculated from the formula

ECNa = 0.89 (82 Br space) (serum sodium concentration)

This formula is derived from the data presented by Moore et al (53) These authors determined total extracellular sodium from the formula

$$ECNa = (\text{Serum sodium conc.}) \left(PV + \frac{0.95}{0.92}(ECW - PV)\right),$$

where the factor 0 92 corrects for plasma solids, and the factor 0 95 corrects for the Donnan effect of cations across capillary membranes. A correction of Br space with a constant of 0 89 was chosen for the present series, since this factor in normal individuals and in edematous and nonedematous cardiac subjects predicted the same average values for ECNa as obtained by the formula given above.

e Residual sodium

The residual sodium, i. e the part of Na_e not accounted for in terms of total extracellular sodium is obtained from the formula

$$Residual\ Na = Na_e - ECNa.$$

Residual sodium represents the sum of intracellular sodium and exchangeable bone sodium outside the extracellular phase.

Table II. Body composition in patients with heart disease

Case no.	Na_e (mEq)	Cl_e (mEq)	ECW (l)	ECNa (mEq)	Residual Na (mEq)	K_e (mEq)	BCM (kg)	ECS + Fat (kg)	$Na_e + K_e$ (mEq)	Na_e/K_e (mEq/mEq)
Edematous heart disease										
Males										
1	4,858	3,167	28.4	4,204	654	2,562	21.3	13.3	7 420	1.59
2	3,520	2,727	23.2	3,360	160	2,514	20.9	20.9	6,034	1.40
3	5,795	4,139	36.7	5,358	437	1,815	15.1	24.0	7,610	3.19
4	3,991	2,603	23.3	3,302	688	2,446	20.3	24.4	6,437	1.63
5	4,465	3,504	30.4	4 410	55	2,519	20.9	13.7	6,984	1.77
6	4,533	3,104	27.0	3,822	711	2,033	16.9	24.4	6,566	2.23
7	3,203	2,075	18.6	2,746	457	2,117	17.6	21.5	5,320	1.51
8	4,107	3,012	27.3	3,694	413	2,420	20.1	24.4	6,527	1.70
9	3,084	2,139	20.6	2,854	230	2,195	18.2	21.2	5,279	1.41
10	4,691	3,202	31.5	4,368	323	1,834	15.2	26.6	6,525	2.56
11	6,368	4,880	46.6	6,170	198	2,880	23.9	28.9	9,248	2.21
12	3,204	2,173	19.7	2,836	368	2,788	23.1	20.7	5,992	1.15
Nonedematous heart disease										
Males										
13	3,509	2,278	20.6	3,060	449	3,118	25.8	25.0	6,627	1.13
14	3,074	2,100	19.0	2,778	296	2,742	22.8	20.6	5,816	1.12
15	2,917	1 701	15 6	2,300	617	2,306	19.1	21.6	5,223	1.26
16	2,434	1,708	17.0	2,341	93	2,216	18.4	24.1	4,650	1.10
17	2,735	1,80[illegible]	16.3	2,501	234	2,843	23.6	33.1	5,578	0.96
18	2,787	1,849	17.3	2,417	370	1,982	16.5	21.0	4,769	1.40
19	4,295	2,950	25.4	3,708	587	3,560	29.5	30.7	7,855	1.21
20	3,[illegible]	2,647	22.1	3,273	223	3,437	28.7	24.7	6,957	1.02
21	3,754	2,417	20 4	3,060	694	4,132	34.3	21.0	7,886	0.91
22	2,933	2,080	17.2	2,510	423	3,129	26.0	28.3	6,062	0.94
23	2,886	1,895	15.8	2,378	508	3,192	26.5	20.7	6,078	0.90
24	3,305	2,350	19.4	2,887	418	2,985	24 8	25.1	6,290	1 11
Edematous heart disease										
Females										
25	3,714	2,370	23.1	3,322	392	1,670	13 9	13.5	5,384	2.22
26	3,236	2,098	18.7	2,793	443	1,666	13.8	24.8	4,902	1.95
27	3,736	2,642	23.7	3,406	330	1,870	15.3	21.3	5,606	2.00
28	3,263	2,188	18 9	2,822	441	1,921	15.9	18.7	5,184	1.70
29	5,683	4,026	36.3	5,339	344	2,421	20 1	20.1	8,104	2.35
30	3 463	2,533	22.3	3,257	206	1,098	9 1	17.0	4,561	3.15
31	2,431	1,739	15.1	2,137	294	1,725	14.5	16.9	4,376	1 42
32	4,293	2,917	28.3	3,930	363	2,294	19.0	17.1	6,587	1.87
33	2,905	1,899	17.3	2,470	435	1,180	9.8	30.4	4,085	2.46
34	2,760	1,734	17.3	2,548	212	1,504	12.5	16.5	4,264	1.84
35	2,582	1,763	15.1	2,200	382	1,642	13.6	12.0	4 224	1.57
36	3,456	2,231	24 9	3,302	154	1,030	8.5	24.3	4 486	3.36

Table II (cont.)

Case no.	Na_e (mEq)	Cl_e (mEq)	ECW (l)	ECNa (mEq)	Residual Na (mEq)	K_e (mEq)	BCM (kg)	ECS + Fat (kg)	$Na_e + K_e$ (mEq)	Na_e/K_e (mEq/mEq)
Nonedematous heart disease										
Females										
37	2,096	1,981	16.4	2,363	333	1 726	14.3	27.3	4,422	1.56
38	1 767	1,333	11 4	1,615	152	1,587	13.2	13.4	3,334	1.11
39	1,9.8	1,396	12.1	1,827	131	1,926	16.0	16.4	3,834	1.02
40	2,536	1 733	14.2	2,087	469	2,403	19.9	23.2	4,939	1.06
41	2,472	1,864	15.6	2,302	169	2,143	17.8	20.7	4,617	1.13
42	1,981	1 404	12.0	1 761	220	1,360	11.3	30.0	3,341	1.46
43	2 642	1,916	18.1	2,500	142	2,409	19.2	20.7	5,051	1.10
44	..033	1 407	12.5	1 780	253	1 477	12.3	20.8	3,510	1.38
45	2,011	1 187	12.1	1 777	234	1 685	14.0	20.4	3,696	1.19
46	2 432	1,678	14.7	2,098	334	1 731	14 4	28.7	4,183	1.42
47	2 684	1,875	15.7	2,300	384	,255	18.7	21 4	4,939	1.19
48	2,421	1 793	14.9	2,202	219	2,091	17 4	22.2	4,512	1.16

Table III Mean values (± SE) of age, body weight and serum electrolyte concentrations in heart disease

	No.	Age (yrs)	Body weight (kg)	Body height (cm)	Serum Na (mEq/l)	Serum Cl (mEq/l)	Serum K (mEq/l)
Males							
Edematous	12	45.0 ± 3.56	69.3 ± 3.16	173.8 ± 1.39	137.3 ± 1.36	95.3 ± 1.22	4.24 ± 0.14
Nonedematous	12	44 4 ± 2.67	68.2 ± 2.64	173.6 ± 1.90	141.8 ± 1.21	97.8 ± 1.66	4.46 ± 0.10
Difference		+ 0.6 ± 4.52	+ 1 1 ± 4 12	+ 0.2 ± 2.35	− 4.5 ± 1.82	− 2.5 ± 2.06	− 0.22 ± 0.15
Signific. of diff.		—	—	—	*	—	—
Females							
Edematous	12	47.5 ± 3.01	54.9 ± 2.71	164.0 ± 2.57	140.0 ± 1.30	94.8 ± 1.96	4.36 ± 0.14
Nonedematous	12	48.0 ± 2.75	52.1 ± 1 96	159.6 ± 1.83	140.3 ± 0.94	99.2 ± 1.78	4.42 ± 0.14
Difference		− 0.5 ± 4.08	+ 2.8 ± 3.35	+ 4 4 ± 3.16	− 0.3 ± 1.61	− 4.4 ± 2.65	− 0.06 ± 0.19
Signific. of diff.		—	—	—	—	—	—

Table IV Statistical summary of body composition in edematous and nonedematous males and females with heart disease

	24 N space (l)	Na_e (mEq)	82 Br space (l)	Cl_e (mEq)	ECW (l)	ECNa (mEq)	Residual Na (mEq)	K_e (mEq)	BCM (kg)	ECS + Fat (kg)	$Na_e + K_e$ (mEq)	Na_e/K_e (mEq/mEq)
Males												
Edematous	31.6 ±2.33	4,516 ±297	32.3 ±2.68	3,069 ±240	27.8 ±2.30	3,927 ±303	391 ±62	2,344 ±100	19.4 ±0.82	22.1 ±1.26	6,662 ±313	1.86 ±0.17
Non-edematous	22.4 ±1.01	3,178 ±149	21.9 ±0.98	2,148 ±113	18.8 ±0.84	2,768 ±127	410 ±51	2,972 ±176	24.7 ±1.46	24.7 ±1.20	6,150 ±305	1.09 ±0.04
Difference	+8.2 ±2.54	+1,140 ±333	+10.4 ±2.85	+821 ±266	+9.0 ±2.45	+1,159 ±378	−19 ±80	−628 ±202	−5.3 ±1.68	2.6 ±1.74	+512 ±437	+0.77 ±0.17
Signific. of diff.		**					—		*	—	—	
Females												
Edematous	24.8 ±1.74	3,463 ±233	25.1 ±2.00	2,374 ±108	21.6 ±1.72	3,131 ±233	333 ±28	1,606 ±125	13.8 ±1.04	19.5 ±1.49	5,131 ±336	2.16 ±0.17
Non-edematous	16.5 ±0.71	2,306 ±98	16.4 ±0.71	1,631 ±78	14.1 ±0.81	2,051 ±84	255 ±31	1,899 ±103	15.7 ±0.82	22.3 ±1.40	4,205 ±184	1.23 ±0.04
Difference	+8.3 ±1.88	+1,157 ±270	+8.7 ±2.12	+743 ±204	+7.5 ±1.62	+1,080 ±298	+78 ±42	−231 ±162	−1.9 ±1.33	−2.8 ±2.03	+926 ±383	+0.93 ±0.18
Signific. of diff.							—	—	—	—		

f Total exchangeable cation ($Na_e + K_e$)

Since sodium is essentially an extracellular electrolyte, and potassium is confined mainly to the cells, the sum $Na_e + K_e$ is of interest as measure of the large majority of cations in the w terholding tissues of the body

g. Na_e K_e *ratio*

It follows from the preceding section that the Na_e K_e ratio may appear to be sensitive index of distortion of the extracellular-intracellular relationships

Predicted normal values

I the analyses to follow the results of body composition measurements obtained in patients will often be compared with predicted normal values. These group analyses are based upon prediction of normal body composition for each individual using the data presented by Moore et al. (53) and taking into account the effect on body composition of sex, age, and body weight.

The use of the data for normal body composition given by Moore et al. (53) appears to be justified by the results obtained with the present technique in 11 adult normal individuals. When the values obtained are expressed as percentage of predicted normal values, the mean results (± s. d.) are as follows Cl_e 100.4 ± 8.6 %, Na_e 102.5 ± 7.5 %, ECW 102.3 ± 8.1 %, ECNa 104.4 ± 8.7 %, residual Na 86.9 ± 36.8 %, K_e 103.8 ± 12.3 %, (ECS + Fat) 96.9 ± 14.8 %, $Na_e + K_e$ 104.0 ± 9.6 %, Na_e K_e ratio 99.9 ± 8.2 %

Statistical methods

Conventional statistical methods are used (69) I group comparisons with a significant

Table I Statistical summary of body composition mean values (± SE) of edematous male and female cardiac patients compared with predicted normal values

	Na_e (mEq)	Cl_e (mEq)	ECV (l)	ECNa (mEq)	Residual Na (mEq)	K_e (mEq)	BCM (kg)	ECS + Ft (kg)	$Na_e + K_e$ (mEq)	Na_e/K_e (mEq/mEq)
Males										
Edematous subjects	4,318 ±297	3 069 ±240	27.8 ±2.30	3,927 ±303	391 ±62	2,344 ±100	19.4 ±0.82	22.1 ±1.26	6,662 ±313	1.86 ±0.14
Predicted normal	2,863 ±100	2,056 ±55	16.7 ±0.42	2 440 ±46	425 ±20	3 132 ±116	26.0 ±0.96	26.6 ±2.06	5,997 ±173	0.92 ±0.02
Difference	+1 455 ±314	+1 013 ±247	+11 1 ±2.34	+1 487 ±306	−34 ±65	−788 ±153	−6.6 ±1.27	−4.5 ±2.42	+665 ±357	+0.94 ±0.17
Signific. of diff	***	***	***	* *	—	* *	**	—	—	***
Females										
Edematous subjects	3 463 ±255	2,374 ±188	21.6 ±1 7?	3 131 ±253	333 ±?8	1,668 ±125	13.8 ±1.04	19.5 ±1 49	5,131 ±336	2.16 ±0.17
Predicted normal	2 19 ±49	1 630 ±31	1 .6 ±0.36	1,861 ±32	334 ±17	2,116 ±45	17.6 ±0.37	24 7 ±1.97	4,311 ±94	1.03 ±0.02
Difference	+1,268 ±258	+744 ±191	+9.0 ±1 6	+1,.70 ±255	−1 ±33	−448 ±133	−3.8 ±1 10	−5.2 ±2.49	+820 ±349	+1 13 ±0.17
Signific of diff	***	*	***	*	—	**	**	*	*	**

difference in variance the t test was evaluated with the degrees of freedom of (n—1) instead of 2 (n—1)

The significance levels of differences are indicated by hyphens (—) or asterisks (*) with the following code

— not significant
* p is less than 0.05, but more than 0.01
** p is less than 0 01 but more than 0 001
*** p is less than 0 001

Results

The clinical and laboratory data for the total series are given in tables I and II A statistical comparison of mean values for edematous and nonedematous subjects of both sexes is shown in tables III and IV Comparison with predicted normal values are summarized in tables V and VI

Consideration of individual values and mean results lead to the following conclusions

I Total exchangeable electrolytes and their volumes of dilution

a. Total exchangeable potassium (K_e)

It appears from table II and from fig 1 and 2 that K tends to be higher in male than in female cardiac patients similar to the pattern found in normal subjects (53 66) In both sexes a considerable degree of overlapping of values between

Table VI Statistical summary of body composition mean values (± SE) of nonedematous male and female cardiac patients and predicted normal values

	Na_e (mEq)	Cl_e (mEq)	ECW (l)	ECN (mEq)	Residual Na (mEq)	K_e (mEq)	BCM (kg)	ECS + fat (kg)	$Na_e + K_e$ (mEq)	Na_e/K_e (mEq/mEq)
Males										
Nonedematous subjects	3,178 ±149	2,148 ±113	18.8 0.84	2,768 ±127	410 ±51	2,972 ±176	24 7 ±1 46	24.7 ±1.20	6,150 ±305	1.09 ±0.04
Predicted normal	2,847 ±55	2,040 ±45	16.6 ±0.33	2,421 ±38	418 ±17	3,147 ±83	26.1 ±0.71	25.5 ±1.81	5,994 ±132	0.91 ±0.02
Difference	+331 ±159	+108 ±122	+2.2 ±0.91	+347 ±135	−8 ±54	−175 ±195	−1 4 ±1.62	−0.8 ±2.17	+156 ±332	+0.18 ±0 03
Signific. of diff.		—			—	—	—	—	—	
Females										
Nonedematous subjects	2,306 ±96	1,631 ±78	14.1 ±0.61	2,051 ±84	255 ±31	1,899 ±103	15.7 ±0 82	22.3 ±1 40	4,205 ±184	1.23 ±0.04
Predicted normal	2,145 ±33	1,596 ±22	12.2 ±0.26	1,830 ±23	315 ±13	2,033 ±42	16.9 ±0.35	23.0 ±1 43	4,178 ±72	1.06 ±0.02
Difference	+161 ±102	+35 ±81	+1.9 ±0.66	+221 ±87	−60 ±34	−134 ±111	−1.2 ±0.90	−0.7 ±2.02	+27 ±197	+0.17 ±0.03
Signific. of diff.		—			—	—	—	—	—	

edematous and nonedematous patients is also noted.

The mean value for K in edematous males (2,344 mEq) is significantly lower than the mean of nonedematous males (2,972 mEq) Similarly the mean value in edematous females (1 668 mEq) is lower than the mean of nonedematous females (1 899 mEq) although statistical significance is not achieved (table IV)

In comparison with predicted normal values K_e is significantly lowered in edematous groups of both sexes (table V and fig 1)

In nonedematous groups the mean values for K_e are slightly but not significantly lower than predicted normal values (table VI and fig 2)

b Total exchangeable sodium (Na_e) *and 24 Na space*

The established relationship between edema formation and increased body sodium content is confirmed by the measured values for Na given in table II and in fig 3

The mean value for Na_e in edematous males (4,318 mEq) is significantly higher than the mean of the nonedematous group (3 178 mEq) Similarly the mean value in edematous females (3 463 mEq) is significantly higher than the mean of nonedematous females (2,306 mEq) (table IV)

In edematous groups of both sexes the mean values for Na_e are significantly

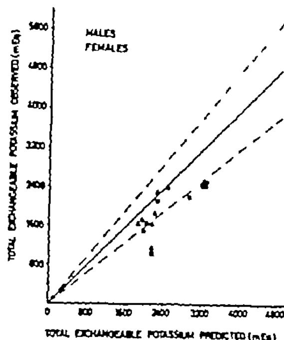


Fig 1 Relationship of observed and predicted normal values of total exchangeable potassium in edematous males and females with heart disease. The dotted lines indicat the 95 con fidence limits of predicted normal values.

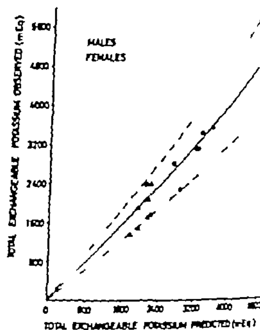


Fig 2 Relationship of observed and predicted normal values for total exchangeable potassium in nonedematous males and females with heart disease. The dotted lines indicate the 95 confidence limits of predicted normal values.

higher than predicted normal values as shown in table V and fig 3

In nonedematous groups of both sexes the mean values for Na_e are slightly but not significantly higher than predicted normal values (table VI)

The 24 Na space follows closely the pattern of Na_e (table IV)

c Total exchangeable chloride (Cl_e) *and* 82 Br *space*

The values for Cl and 82 Br space given in table II and in fig 4 confirm the established relationship between edema formation and increased body chloride and increased bromide space.

The mean value for Cl in edematous males (3 069 mEq) is significantly higher than the mean of the nonedematous males (2 148 mEq) Similarly the mean of Cl_e in edematous females (2,374 mEq) is significantly higher than the mean of nonedematous females (1 631 mEq) (table IV)

In edematous groups of both sexes the observed Cl_e is significantly higher than predicted normal values (table V and fig 4)

The 82 Br space follows the pattern of Cl (table IV)

d. Correlations of 24 Na *space and* 82 Br *space and of* Na *and* Cl

As shown in fig 5 a strikingly high correlation is found between 24 Na space and 82 Br space ($r = 0.98$, $p < 0.001$) Similarly a highly significant correlation is present between Na_e and Cl ($r = 0.97$ $p < 0.001$) as depicted in fig 6. In regression analysis of both groups of

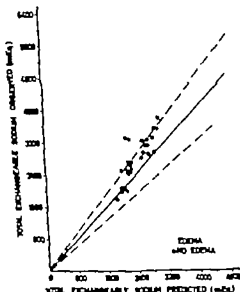


Fig. 3. Relationship of observed and predicted normal values for total exchangeable sodium in edematous and nonedematous subjects with heart disease. The dotted lines indicate the 95 confidence limits of predicted normal values.

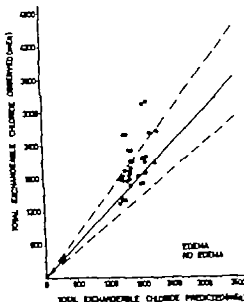


Fig. 4. Relationship of observed and predicted normal values for total exchangeable chloride in edematous and nonedematous subjects with heart disease. The dotted lines indicate the 95 % confidence limits of predicted normal values.

variables it was found that the slopes of regression lines were not significantly different in edematous and nonedematous groups. These findings are most easily interpreted on the assumption that sodium and chloride in cardiac edema are increased being together in an expanded extracellular phase.

Total extracellular water (ECW) *total extracellular sodium* (ECN) *and residual sodium*

It follows from the preceding analysis of Br space and Cl that the derived values for ECW must be higher in edematous than in nonedematous groups.

Accordingly it appears from table IV that the mean value for ECW in edematous males (27.8 l) is significantly higher than the mean of the nonedematous males (18.8 l) Similarly the mean value in edematous females (21 6 l) is significantly higher than the mean of nonedematous females (14 1 l)

In both sexes the mean values obtained for ECW in edematous groups are significantly higher than predicted normal values (table V)

In nonedematous groups the mean values for ECW are slightly but not significantly higher than predicted normal values (table VI)

The total extracellular sodium (ECNa) derived from ECW and serum sodium concentration, follows the pattern of ECW as shown in tables IV V and VI.

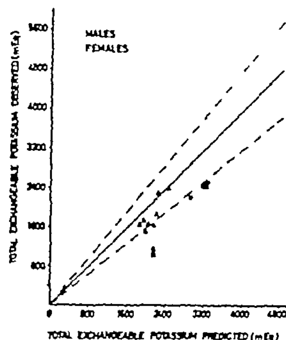


Fig 1 Relationship of observed and predicted normal values of total exchangeable potassium in edematous males and females with heart disease. The dotted lines indicate the 95 con fidence limits of predicted normal values.

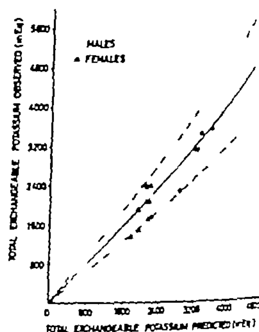


Fig 2 Relationship of observed and predicted normal values for total exchangeable potassium in nonedematous males and females with heart disease The dotted lines indicat the 95 % confidence limits of predicted normal values.

higher than predicted normal values as shown in table V and fig 3

In nonedematous groups of both sexes the mean values for Na_e are slightly but not significantly higher than predicted normal values (table VI)

The 24 Na space follows closely the pattern of Na (table IV)

c Total exchangeable chloride (Cl_e) *and* 82 Br *space*

The values for Cl_e and 82 Br space given in table II and in fig 4 confirm the established relationship between edema formation and increased body chloride and increased bromide space

The mean value for Cl in edematous males (3 069 mEq) is significantly higher than the mean of the nonedematous males (2 148 mEq) Similarly the mean of Cl_e in edematous females (2 374 mEq) is significantly higher than the mean of nonedematous females (1 631 mEq) (table IV)

In edematous groups of both sexes the observed Cl is significantly higher than predicted normal values (table V and fig 4)

The 82 Br space follows the pattern of Cl (table IV)

d. Correlations of 24 Na *space and* 82 Br *space and of* Na *and* Cl_e

As shown in fig 5 a strikingly high correlation is found between 24 Na space and 82 Br space ($r = 0.98$ $p < 0.001$) Similarly a highly significant correlation is present between Na_e and Cl_e ($r = 0.97$ $p < 0.001$) as depicted in fig 6 In regression analysis of both groups of

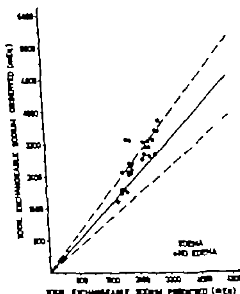


Fig. 3. Relationship of observed and predicted normal values for total exchangeable sodium in edematous and nonedematous subjects with heart disease. The dotted lines indicate the 95 % confidence limits of predicted normal values.

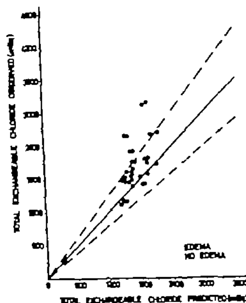


Fig 4 Relationship of observed and predicted normal alues for total exchangeable chloride in edematous and nonedematous subjects with heart disease. The dotted lines indicate the 95 % confidence limits of predicted normal values.

variables it was found that the slopes of regression lines were not significantly different in edematous and nonedematous groups. These findings are most easily interpreted on the assumption that sodium and chloride in cardiac edema are increased being together in an expanded extracellular phase.

Total extracellular water (ECW) *total extracellular sodium* (ECNa) *and residual sodium*

It follows from the preceding analysis of Br space and Cl_e that the derived values for ECW must be higher in edematous than in nonedematous groups.

Accordingly it ppears from table IV that the mean value for ECW in edematous males (27.8 l) is significantly higher than the mean of the nonedematous males (18 8 l) Similarly the mean value in edematous females (21 6 l) is significantly higher than the mean of nonedematous females (14 1 l)

In both sexes the mean values obtained for ECW in edematous groups are significantly higher than predicted normal values (table V)

In nonedematous groups the mean values for ECW are slightly but not significantly higher than predicted normal values (tabl VI)

The total extracellular sodium (ECNa) derived from ECW and serum sodium concentration, follows the pattern of ECW as shown in tables IV V and VI.

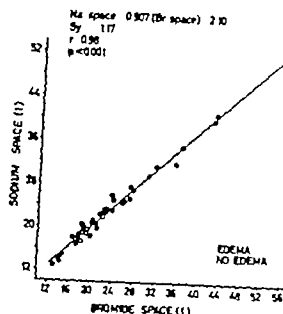


Fig 5 Relationship of sodium space to bromide space in heart disease

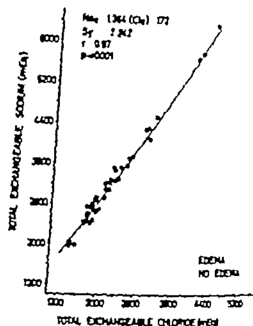


Fig 6. Relationship of total exchangeable sodium to total exchangeable chloride in heart disease

The residual sodium i e the part of Na not accounted for in terms of extracellular sodium presents a mean value of 391 mEq in edematous males and of 410 mEq in nonedematous males and a mean of 333 mEq in edematous females and of 255 mEq in nonedematous females None of these differences is statistically significant Similarly the predicted normal values for residual sodium are very close to the obtained results in all four groups as shown in tables V and VI In this study no significant evidence of a storing away of sodium outside the extracellular phase was found

f Total exchangeable sodium + total exchangeable potassium ($Na_e + K_e$) (fig 7)

As shown in table IV the sum $Na_e + K_e$ has a higher mean value in edematous males (6 662 mEq) than in nonedematous males (6 150 mEq) and in edematous females (5 191 mEq) than in nonedematous females (4 205 mEq) Only the latter difference is probably statistically significant but the trend is similar in the two sexes. The tendency to increase of $Na_e + K_e$ in edematous groups is also apparent from the comparison with predicted normal values (table V and fig 7) In nonedematous groups the observed values are very close to predicted normal values (table VI and fig 7)

g Na_e K_e ratio

The trends for increase of Na_e and decrease of K_e in cardiac disease makes the Na_e K_e ratio a highly sensitive index of distortion of body composition in this study

In edematous males the mean value for Na_e K_e ratio (1 86) is significantly higher than the mean of nonedematous

males (1 09) Similarly the mean value of edematous females (2.16) is significantly higher than the mean of nonedematous females (1.23) The same pattern of increased Na_e K ratio in edematous patients is apparent from the comparison with predicted normal values (table V)

In nonedematous groups the observed values for Na_e K_e ratio are significantly higher than predicted normal values ($p < 0.01$) being the only significant evidence of a distorted body composition in this group (table VI)

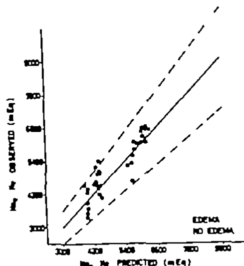


Fig 7 Relationship of observed and predicted normal values for $Na_e + K_e$ in edematous and nonedematous subjects with heart disease. The dotted lines indicate the 95 % confidence limits of predicted normal values.

II Gross partitions of body mass

a. Body mass (body weight)

The mean body mass (body weight) is not significantly higher in edematous than in nonedematous groups of either sex (table III) This tendency is in accordance with the findings in a larger series of consecutively admitted cardiac patients of comparable age and sex groups as shown in table VII

The similarity of mean body mass in edematous and nonedematous groups which is not caused by a deliberate selection of the material is suggestive of loss of body constituents during the cumulation of extracellular edema in the edematous groups. In order to elucidate this problem a partitioning of total body mass will be attempted in the following exposition according to the formula Body mass = body cell mass + extracellular water + (extracellular solids + body fat)

b Body cell mass

Assuming normal intracellular potassium concentration a first order estimate of body cell mass (BCM) may be carried out as shown in table II The mean value for BCM in edematous males (19 4 kg) is significantly lower than the mean of nonedematous males (24 7 kg) Similarly the mean BCM in edematous females (13.8 kg) is lower than the mean of nonedematous females (15 7 kg) but statistical significance is not achieved. The values obtained in edematous groups are significantly lower than predicted normal values in both sexes (table V)

In nonedematous groups the values obtained for BCM are not significantly different from predicted normal values (table VI)

Extracellular water

As shown above ECW is significantly higher in edematous than in nonedematous patients of both sexes, and the values obtained in edematous groups are higher than predicted normal values.

Table VII Body weight in a consecutive series of edematous and nonedematous cardiac patients of comparable age groups

	Edema	Age (Mean ± SE)	Body height (Mean ± SE)	Body weight (Mean ± SE)	No.
Males	+	51 4 ± 2.04	173.0 ± 1.30	73.6 ± 1 72	20
	−	49.3 ± 1.95	175.3 ± 1.56	73.0 ± 2.00	20
Females	+	49.9 ± 2.78	161.0 ± 1.33	59.9 ± 2.23	20
	−	47.3 ± 1 16	160.0 ± 1.20	59.5 ± 1.73	20

Table VIII Equilibration data for total exchangeable sodium, 24 Na space total exchangeable chloride and 82 Br space in edematous heart failure Values expressed as fractions of the 36-hour equilibration values

	Measurement			
	24 hrs (Mean ± SD)	36 hrs	48 hrs (Mean ± SD)	No. of pat.
Total exchangeable sodium	0.99 ± 0.04	1 00	1.01 ± 0.03	14
Sodium space	0.98 ± 0.04	1.00	1.01 ± 0.03	14
Total exchangeable chloride	1.00 ± 0.03	1.00	1.02 ± 0.02	9
Bromide space	1.00 ± 0.03	1.00	1.02 ± 0.02	9

Table IX Equilibration data for total exchangeable potassium in edematous and nonedematous cardiac patients and in normal individuals. Value expressed as fractions of the 96-hour equilibration values

	Period			
	24 hrs (Mean ± SD)	36 hrs	48 hrs (Mean ± SD)	No. of pat.
Edematous cardiacs	0.90 ± 0.06	1.00	1.03 ± 0.05	11
Nonedematous cardiacs	0.91 ± 0.06	1.00	1.03 ± 0.04	10
Normal individuals	0.88 ± 0.06	1.00	1.02 ± 0 05	9

d Extracellular solids and body fat (ECS + fat)

In actual mean values the quantity (ECS + fat) is lower in edematous males (22 1 kg) than in nonedematous males (24 7 kg) and lower in edematous females (19 5 kg) than in nonedematous females (22 3 kg) Similarly the values obtained in edematous groups are lower than predicted normal values (table V) None of these differences are statistically significant but the trends are similar in all comparisons.

In nonedematous patients the values observed differ little from the values predicted (table VI)

Table X. Comparison of alterations in body sodium content measured by isotope dilution techniques and by metabolic balance method. Interval of 7 days

Patient	ΔNa_e (mEq)	Na balance (mEq)
A. N.	− 337	−384
R. L.	− 847	−390
G. H.	− 284	−352
S. I.	− 1,035	−931
L. M.	− 7	− 70

Table XI. Comparison of alterations in total body potassium measured by metabolic balance method and by isotope dilution studies. Interval of 7 days

Patient	ΔK_e (mEq)	K balance (mEq)
A. N.	+ 68	+12
R. J.	+ 30	+70
T. N.	− 85	−52
R. L.	− 94	−79
E. J.	−231	−24
S. I.	+122	+79

III. Rates of equilibration of isotopes in cardiac edema

The mean results of rates of equilibration of isotopes are shown in tables VIII and IX. After 24 hours the tracers 24 Na and 82 Br are nearly completely equilibrated with body sodium and body chloride in edematous cardiacs.

With the isotope 42 K the total exchangeable potassium determined after 24 hours was less than after 36 hours, and the rise from 36 to 48 hours did not exceed 3 per cent, indicating that almost complete equilibration had taken place during this period. The rates of equilibration after 24 hours, however, were the same in edematous, nonedematous, and normal subjects and allow a comparison of 24 hour values in these groups. Since K determination after 24 hours requires a smaller dose of isotope and is comparable to the normal values given in the literature (53), a 24 hour equilibration period was chosen for all three isotopes in the present study.

IV. Simultaneous metabolic balance, exchangeable sodium and exchangeable potassium measurements

The complete results are tabulated in tables X and XI. The changes in body sodium and potassium as measured with radioisotope dilution techniques were comparable with those observed using metabolic balance method. The maximum difference between values obtained by each method was 237 mEq for sodium and 207 mEq for potassium — less than 7 and 8 per cent, respectively, of the total exchangeable sodium and potassium values.

Discussion

Figs. 1—7 depict the body compositional data of edematous and nonedematous cardiac patients.

The mean body mass (body weight) is slightly but not significantly higher in edematous than in nonedematous groups of both sexes. A similar tendency is noted in a larger series of consecutively admitted patients with heart disease of a similar age range as shown in table VIII. Together with the fact that patients with progressive mitral stenosis in spite of frequent development of edema demonstrate an average weight loss when followed over a decade (58), these observations suggest that expansion of the extracellular phase in heart disease is often accompanied by a loss of other body constituents.

In accordance with this view a direct comparison of body composition of edematous and nonedematous groups reveals significant trends of difference in both sexes in spite of the similarity of mean body weights of the groups compared (table IV) In terms of direct measurements cardiac edema appears to be associated with a decrease of total exchangeable potassium and an increase of total exchangeable sodium 24 Na space total exchangeable chloride and 82 Br space and an increased Na K ratio.

Since the groups compared are separated on clinical grounds according to the presence or absence of edema it must be ensured that the differences obtained are not a reflection of varying efficiency of methods in edematous and nonedematous subjects or due to other unobserved differences. It has been shown that the early phase of equilibration of isotopes with body constituents may be delayed in the presence of edema (62 71) In our experience 24 Na had reached a nearly complete equilibration with total exchangeable sodium in edematous cardiacs after 24 hours and the same finding applied to the equilibration of 82 Br with body chloride (table VIII) With the isotope 42 K the rate of equilibration with K_e after 24 hours was comparable in edematous, nonedematous and normal subjects (table IX) These results are similar to the findings of other investigators (62 70) and permit a comparison of the 24-hour values of total exchangeable electrolytes in edematous nonedematous, and normal subjects Simultaneous measurements of changes in total body sodium and potassium by sequential tracer studies and by metabolic balance methods showed a high degree of accordance (tables X and XI) and militates against the possibility that variations in exchangeability of body electrolytes with isotopes may influence the results (4 11 16) Apparently variations in efficiency of methods cannot account for the differences in composition observed. Since also the systematic influence on body composition of sex (19 41 53 66) age (19, 42 53 66) and body weight (41 42, 53 66) has essentially been ruled out by the selection and grouping of the series, the differences in patterns of composition in edematous and nonedematous cardiacs must apparently be related to variations in the stage of the disease and in medicinal treatment applied as discussed below

The trend for a decrease of total exchangeable potassium and for an increase of total exchangeable sodium total exchangeable chloride and of Na K_e ratio in the presence of edema is confirmed when the values obtained in edematous subjects are compared with predicted normal values (tables V and VI) The use of predicted normal values as a basis of comparison in the present study appears justified by the results obtained in a series of normal subjects studied with the present techniques and takes into account the systematic influence on body composition of sex, age, and body weight (53) A minor error might be introduced in these comparisons by the weight increase caused by accumulated edema resulting in overestimation of normal values and tending to underestimate the increase of sodium and chloride and to overestimate the loss of potassium This error however is partly counterbalanced by the apparent loss of other body constituents as reflected in the slight mean difference of body weight in edematous and nonedematous groups and is not of such a magnitude that it will affect the significance of the differences observed.

The body composition pattern of nonedematous groups differs little from predicted normal values. In both sexes a slight, but insignificant increase of Na_e and Cl_e and decrease of K_e are noted. An elevated Na_e K_e ratio is the only statistically significant evidence of abnormal body composition (table VI)

When Na_e K_e ratio appears to be the most sensitive single index of a distortion in body composition in heart disease with and without edema the explanation is that K_e tends to decrease at the same time as Na_e rises.

Since nearly all body potassium is confined to the cells, and since potassium is the major intracellular cation (44) the decrement of total exchangeable potassium in edematous heart disease is strong evidence that body cell mass is injured in this disease state. A decrease of K_e might be caused by a loss of potassium from the cell mass or by a reduction of total body cell mass. With the methods used in the present study no distinction can be made between these two possibilities. In untreated heart failure histochemical studies of muscle tissue has not revealed any systematic reduction in K.N ratio (48, 72, 74) Diuretic treatment with benzothiadiazines (63) or mercurials (67) may cause potassium loss in heart disease, but it ha also been shown that potassium balance may be maintained under these circumstances when a liberal supply of potassium chloride or spironolactones are added to the regimen (60, 68) Since these precautions were taken in patients included in the present study it would seem a fair assumption that a specific potassium deficiency has played a minor role in the present series. On th other hand, multiple tracer studies in patients with edematous heart disease have demonstrated a reduction in total intracellular water obtained as a difference between total body water and extra cellular water and an almost corresponding decrease of total exchangeable potassium (53) suggesting that a reduction of total cell mass is the major cause of the decrease of K_e in edematous heart disease. It may be presumed therefore, that a reduction of body cell mass also plays the major role for the decrease of total exchangeable potassium in the present series.

Assuming that body cell mass has a normal potassium content in the present series a first order estimate of body cell mass from K_e results in values which are significantly lower in edematous than in nonedematous males, and a similar tendency is found in the females. In both sexes the values obtained in edematous groups are significantly lower than predicted normal values. To the extent that the assumptions basic to the derivations of body cell mass hold true these findings support the contention that cardiac edema is often accompanied by a loss of other body constituents.

While the decrement of K contributes to the rise of Na_e K_e ratio in edematous heart disease the major cause of the rise in this ratio is an increase of Na_e, which is paralleled by a rise in Cl_e, 24 Na space, and 82 Br space Reflecting the increase of these preponderantly extracellular measurements the derived values for total extracellula water are significantly higher in edematous than in nonedematous males and females, and the values obtained in edematous groups are significantly higher than predicted normal values (tables IV and V) The significance of these findings depends upon the validity of the use of the chloride (bromide) space as a reference for calculation of extracellular water Historically the

In accordance with this view a direct comparison of body composition of edematous and nonedematous groups reveals significant trends of difference in both sexes in spite of the similarity of mean body weights of the groups compared (table IV) In terms of direct measurements cardiac edema appears to be associated with a decrease of total exchangeable potassium and an increase of total exchangeable sodium 24 Na space total exchangeable chloride and 82 Br space and an increased Na K ratio

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evidence that chloride was almost exclusively extracellular in skeletal muscle led to its use as a measure of extracellular water (23 32 86) The demonstration that bromide exchanged almost completely with chloride in biological fluids conditioned the use of this analogous anion for the same purpose (8 59, 82) As evidence accumulated that a small amount of chloride entered cells a correction of the chloride (bromide) space as a measure of extracellular water became necessary To circumvent this objection the saccharides, inulin mannitol and sucrose were applied to measurement of extracellular water (36 37 56) It appeared however that these substances only to a limited extent measured the total extracellular phase of dense connective tissue (12 57) bone cartilage, and gastrointestinal contents (19 46) This heterogeneity of the extracellular phase led to irreconcilable differences between intracellular electrolyte concentrations obtained through in vivo multiple tracer studies and tissue analysis In tracer studies including saccharides as a measure of extracellular volume the average intracellular electrolyte concentrations were about 115 mEq/l of potassium, 35 mEq/l of sodium and 25 mEq/l of chloride (14 15 39) while the figures obtained by tissue analysis were approximately 150 mEq/l of potassium 8 mEq/l of sodium, and 1 2 mEq/l of chloride (6 23 32 40 48 72 73 84)

By contrast multiple in vivo tracer studies including bromide space as a measure of extracellular water results in estimates of the average intracellular potassium concentration which are very close to 150 mEq/l in normal and diseased subjects of both sexes (41 53) Although the extracellular water in these studies was derived from bromide space by help of arbitrary corrections which do not eliminate the heterogeneity of all extracellular subphases (19 46) the total extracellular water obtained by this approach seems at present to represent the best approximation to the true total extracellular fluid volume in normal and in diseased subjects (46, 53)

In the present study strikingly high correlations were observed for the total material between 24 Na space and 82 Br space ($r = 0.98$ $p < 0.001$) and between Na and Cl ($r = 0.97$ $p < 0.001$) These findings are most easily interpreted on the assumption that sodium and chloride in cardiac edema are increased being together in an expanded extracellular phase. The significant correlations are not suggestive of an increased intracellular entrance of chloride in severe heart failure as postulated by Flear and Crampton (27) unless it is assumed that chloride and sodium enter cells in the same concentration relationships as found in the extracellular phase. Such a possibility seems remote, however and is inconsistent with the fact that red cell chloride concentration is unchanged in edematous heart disease (25)

When total exchangeable sodium is divided into total extracellular sodium and residual sodium it appears that total extracellular sodium is significantly increased in edematous groups in relation to nonedematous groups as to predicted normal values (tables IV and V) The residual sodium, i. e. the part of Na not accounted for in terms of extracellular sodium is closely equal in edematous and nonedematous groups of both sexes, and the values obtained in cardiac patients with or without edema are equal to predicted normal values. Obviously the increase in total exchangeable sodium is wholly accounted for by increment of

total extracellular sodium alone. This result is in accordance with the general trend found by Moore et al. (53) and by Birkenfeld et al. (7) and agrees with the fact that histochemical studies showed no significant change in intracellular sodium concentration in muscle tissue in cardiac edema (48, 74) We are unable to confirm that sodium is retained in excess of the probable gain of extracellular water in heart disease as suggested by other investigators (10, 21 6) In these studies no measure of extracellular water was given, and, hitherto, a significant trend for a shift of sodium out of the extracellular phase in cardiac disease has not been shown in multiple tracer studies including measure of extracellular volume.

Since the increase of Na_e in cardiac edema exceeds the decrease of K_e the total exchangeable cation ($Na_e + K_e$) is higher in edematous than in nonedematous groups with a probably significant rise in the female group (p 0.05) The same pattern applies to the comparison of values obtained in edematous groups and predicted normal values (table V and fig. 7) Since the sum $Na_e + K_e$ has been shown to be highly significantly correlated to total body water in normal individuals and in edematous subjects (53, 62) the rise of $Na + K_e$ may be taken as evidence of an increased total body water in the edematous groups. By inference it may then be concluded that total body solids are lower in edematous groups than in nonedematous groups

Consistent with the probable decrease of total body solids the quantity (ECS + fat) is lower in edematous than in nonedematous groups in both sexes and the values obtained in edematous groups are lower than predicted normal values (table V) Although the differences obtained are not statistically significant, the similarity of findings in the two sexes supports the validity of the decrement. It should be noted, however that the derivation of the quantity (ECS + fat) according to the formula ECS + fat = B. Wt. — ECW — BCM involves the assumptions basic to the calculations of extracellular water and body cell mass. Of special interest is the hypothetical situation where the estimate of ECW is correct, and the sum of BCM and (ECS + fat) is constant. To the extent that an intracellular potassium loss is present the body cell mass will be underestimated and (ECS + fat) will be overestimated. Consequently if potassium loss is present in cardiac edema the decrement of BCM in cardiac edema may be overestimated and the decrement of (ECS + fat) may be larger than indicated in the tables. Little is known of the decrement in total noncellular solids (ECS) in disease states, but it is known that body fat may decrease in chronic illness (13) and it would appear a fair assumption that body fat participates in the decrement observed supporting the contention that cardiac edema is often accompanied by a loss of body constituents outside of the extracellular phase. Exact measurement of the fat loss, however must a wait the development of a practical method for determination of total body fat.

The pattern of decreased total exchangeable potassium, decreased body cell mass and probable decrease of body fat found in edematous cardiacs is very similar to the findings described in patients with chronic wasting illness (53) and in starvation (83) and this change in body composition may be interpreted as a consequence of chronic illness and reduced caloric intake. When the evidence of tissue-wasting is more pronounced in edematous than in nonedematous

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groups in the present series it may simply be due to the facts that the edematous groups represent a more advanced stage of disease and present a longer average history of symptoms and signs of heart disease.

It is noteworthy that this alteration in body composition is reversible. It has been shown that successful surgical repair of the basic heart lesion may be followed by restoration of a normal total exchangeable potassium a normal body cell mass and increase of body fat at the same time as the extracellular expansion disappears (53 85)

Summary

Total exchangeable potassium (K) total exchangeable sodium (Na_e) 24 Na space, total exchangeable chloride (Cl) and 82 Br space were measured in 48 cardiac patients with the use of 42 K 24 Na and 82 Br as tracers.

The material comprised 12 edematous and 12 nonedematous subjects of either sex comparable with respect to age and not significantly different in body weight. Comparisons of measured and derived values for body composition in edematous and nonedematous groups and between patient groups and predicted normal body composition revealed that cardiac edema was associated with a decrease of K body cell mass, and probably body fat and with an increase of Na 24 Na space Cl 82 Br space, extracellular water and extracellular sodium derived from Br space and an increased Na K_e ratio The increase of Na_e was accounted for by increment of extracellular sodium alone.

The pattern in body composition in nonedematous patients differed little from predicted normal values except for a significant rise of Na K ratio

For the total series strikingly high correlations between 24 Na space and 82 Br space, and between Na and Cl_e were obtained

Evidence is presented that the differences observed between edematous and nonedematous groups were not caused by differences in rates of equilibration of isotopes or by variations in exchangeability of electrolytes with tracers.

Acknowledgements

This study was supported by a grant from Statens almindelige Videnskabsfond, Copenhagen.

I wish to thank Dr Troels Munkner and Dr Sven Sol sten Sorensen, for generous help with radioactive measurements, and I express my gratitude to Dr Poul Astrup, The Central Laboratory and to Mrs. Vermehren, chief dietician of Medical Department A, for help in balance studies.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Acute Tubular Necrosis Consequent to Encephalomyopathic Thyroid Crisis

By

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The medical thyroid crisis has recently been studied in a number of cases. The critical state may be precipitated by a number of factors which sometimes seem not to be directly related to the thyroid function, i.e. infections, traumata, mental stresses etc. In some cases no precipitating circumstances are recognised and in another group the crisis seems to be induced by changes in treatment for hyperthyroidism. The clinical picture is based on the signs induced by changes in those organs or groups of organs that fail a) neurocerebral acute thyrotoxic encephalomyopathy of W Idenström (33)) b) cardiac,) gastrointestinal d) hepatorenal and e) suprarenal. And lastly there is one type of medical crisis which may be called merely general crisis as it is not possible to point out any specific organ creating the syndrome but the patient just seems to be burning down (29)

In previous studies two cases were observed which apparently recovered from the crisis itself but soon succumbed in renal failure (29)

Since the occurrence of acute tubular necrosis in thyroid crisis seems not to have been specifically mentioned, it seemed to be of interest to report the following case

Case report

The patient, male married lumberer 31 suffered from some kind of infantile encephalomyelitis at the age of 3 1/2 years which left slightly impaired muscular function in the left arm and leg. Apart from measles in the childhood he had been healthy No abuse of alcohol he had stopped tobacco smoking in 1957

In 1955 he got spells of aches behind and around the eyes. The spells lasted from 1 to 2 days and appeared at the beginning at 3 to 4 days intervals Later on the periods in between became longer and in 1959 the attacks appeared with few months intervals.

During the first half-year in 1959 the patient started to feel warm, at times he transpired excessively he was thirsty he had often increased bowel movements, at times he felt

Submitted for publication August 19, 1963.

unusually irritated on effort he had some tremor in the hands. In the autumn 1959 he started to feel some weakness and tiredness in the muscles. He slept well he did not lose weight and he had no heart complaints. In summer 1959 the left eye started to squint and soon thereafter his family observed that it was bulging. Not very much later the same happened to the right eye and the patient got diplopia. In ophthalmological examinations made elsewhere the left eye deviated laterally and the right eye upwards laterally later downwards

On October 7 1959 he was admitted to the Neuropsychiatric Department of Helsinki University. The physical examination revealed a moist warm skin the patient evidently transpired markedly and there was some tremor in the fingers of variable extent. The pulse varied between 70 and 90 beats/min. BP was 140/90

Except for some hemiparetic signs from the left extremities due to the disease mentioned nothing unusual was observed on general neurological examination. Electromyography from the left biceps was entirely normal. Electroencephalography and pneumoencephalography gave normal findings. X ray examination of the skull, the neck and the lungs were normal.

There was marked ophthalmoplegia. The right eye looked straight horizontally and did not move in any direction. The mobility of the left eye was greatly restricted but the eye moved somewhat better especially laterally and upwards. There was very slight irritation of the conjunctivae no chemosis, and slight oedema in the upper lids. No lid retraction or lid lag was present. The thyroid appeared normal in size and consistency and there were no nodules.

Laboratory examinations The blood picture was normal. PBI varied from 9 7 to 7 1 μg/100 ml serum cholesterol was 220 mg/100 ml. The urinary excretion of radioactive iodine was 17.3 % of the dose during the first 24 hours and 1 4 during the next 24-hour period. The 24-hour conversion ratio was 70 %, and the level of PB^{131}I at 72 hours 0.5 %/l. The BMR was + 10 The excretion of 17-ketosteroids was 13 8 mg/day and that of total 17-hydroxylated steroids 9 4 mg/day. Blockade of the stellate ganglion did not affect the eyes.

In an attempt to influence the eye condition which appeared to be mainly either neural or myogenic in origin, prostigmine was given in increasing doses from 90 mg to 120 mg daily for two weeks. As this treatment was stopped the patient felt very tired and Mestinon® was given at first in doses of 10 mg three daily later 60 mg thrice daily. On October 11 additional treatment, with dexamethasone 1 mg thrice daily was started.

On October 27 the patient had some difficulty in swallowing and speaking which, however subsided rapidly. During the next few weeks there was a marked deterioration of the condition. The thyrotoxicosis now became more evident clinically the pulse rate was 110 beats/min., he transpired excessively he felt tired and he appeared jerky. The tremor increased. The thyrohypophysial signs increased rapidly to a IV grade thyro-hypophysical syndrome of early malignant exophthalmos (27 28) with marked irritation of the conjunctivae chemosis, epiphora and large swellings in the lids and around the eyes. The exophthalmometer readings were 21 0 mm on the right, and 23.0 mm on the left eye (Hertel) In addition a marked myasthenic condition developed in spite of continuous Mestinon treatment. He could not raise himself from sitting position and had difficulties in moving on the stairs.

As it appeared that insidiously a marked hyperthyroid state had developed within a very short time with malignant exophthalmos and marked myasthenic signs, the patient as transferred to the First Medical Department of Helsinki University on November 11

On physical examination he appeared markedly restless, he transpired more than usually there was a flush in the face and the skin was moist and warm. The general condition was not very good, he was tired and felt some weakness in the legs. He was thirsty. The pulse rate was 80/min., BP 165/105. There was a soft systolic murmur over the heart and the second sound at the point of Erb was accentuated. There was some weakness on standing on the toes and on pressing the hands; the reflexes were fast. The axillary temperature was 36.5 C. Eye signs as described

Laboratory examinations ESR (Westergren) 14 mm/h, Hb 15.0 g/100 ml, RBC 5.1 mill./mm³ MCH 30, WBC 8,600/mm³ Differen-

tation segm. neutr 48.5 ,, rods eosin. 1.5 %, bas 1.0 % monocytes 14.0 % lymph. 35 %. Total count of eosinophils 112/mm³ PBI 10.6 μg/100 ml, cholesterol 245 mg/100 ml. The ratio of plasma creatine/plasma creatinine was elevated (26) 2.16/0.64 = 3.4 BMR + 25 ,, Urine spec. gravity 1.035. ECG was normal.

Although most of the signs and symptoms resembled moderately severe hyperthyroidism, the insidious development and the fast deterioration along with the muscular features suggested that storm was impending. Hence treatment with potassium iodide (300 mg/day) and carbimazol (Tyrazol® Star 30 mg/day) was immediately instituted. Anabolic steroids (Durabolin® Organon) were also given in doses of 50 mg/day. The Mestinon® treatment was discontinued, as all signs including the negative electromyographic pattern suggested thyrotoxic myopathy rather than myasthenia gravis.

On the next day (Nov. 14) the patient had some difficulties in speaking and in swallowing. The muscular weakness had increased considerably. He was restless and transpired more than previously. This development suggested an impending storm, and intravenous iodine treatment with 2 ml Entodon® (Bayer) was given daily and 100 mg hydrocortisone in glucose infusion daily. Vitamin B_{12} was given during the next four days in doses of 1,000 μg day. The Mestinon treatment was again re-instituted (60 mg 4 times daily). Furthermore, vitamin E (tocopherol acetate, Evitol® Orion) was given for two days in doses of 400 mg day in an attempt to influence the muscular condition. In addition penicillin and streptomycin were administered.

On Nov. 16 the axillary temperature rose to 38.5 C and the condition was now that of medical crisis. The most prominent features were the signs of pseudobulbar palsy and muscular weakness similar to that seen in the acute thyrotoxic encephalomyopathy (53). In addition the eye signs increased considerably, the chemosis and the swellings around the eyes and the bulging increased. He appeared very restless. Laboratory examinations on Oct. 17 showed WBC 13,000 ESR 28 mm/h, CO_2-combining power 63 vol. %. On the 17th at night he was given 0.5 mg reserpine orally owing to the restlessness. On the 17th the Mestinon treatment was again discontinued.

On Nov. 18 in the morning he was bluish-pale in the face and had obvious difficulties in swallowing, speaking and breathing. This condition developed very soon into preagonal state with marked cyanosis and very infrequent respiration along with loss of consciousness. The axillary temperature was 36.5 C, and the BP (systol.) 75 mm Hg. He was given prostigmine 0.5 mg intramuscularly and artificial respiration, and was then transferred to the Aurora Hospital for respirator treatment. When tracheotomy was made for that purpose, the patient made such resistance that curare had to be administered. Evidently the dose of prostigmine had improved the myasthenic condition appreciably. Artificial respiration with the respirator was begun and was continued for the next 10 days. The treatment with intravenous iodine (Entodon®) in doses of 5—6 ml a day was continued for the next 7 days, after which the dose was reduced to 1—2 ml day.

On Nov. 19 the temperature was 39° C and the patient transpired excessively. A marked oliguria was observed (180 ml/day) which became even more pronounced during the next few days (down to 50 ml/day). The specific gravity of the urine decreased to 1.012 and the suspicion of acute renal failure was confirmed during the next few days. The urinary sediment was normal but there was slight proteinuria.

Laboratory examinations (Nov. 19): Hb 13.3 g/100 ml, RBC 4.4 mill/mm³ WBC 12,100/mm³ differentiation neutrophils 71.0 % rods 9.5 %, Eos — Bas — monocytes 6.5 %, lymphocytes 13.0 %. Thrombocytes normal. There was granulation both fine and somewhat coarse, in the leucocytes. Blood-sugar 110 mg/100 ml, NPN 49 mg/100 ml, chlorides 98 mEq/l, potassium 3.9 mEq/l, sodium 136 mEq/l, CO 26.3 mEq/l, proteins 6.0 g/100 ml, haematocrit 58 vol. %. Excretion of coproporphyrins 38 μg/day.

Treatment for acute renal failure with hypertonic glucose and correction of the acidosis with bicarbonate were started. The intravenous treatment with hydrocortisone was discontinued. After 4 days some red cells were observed in the urine and 2 days later

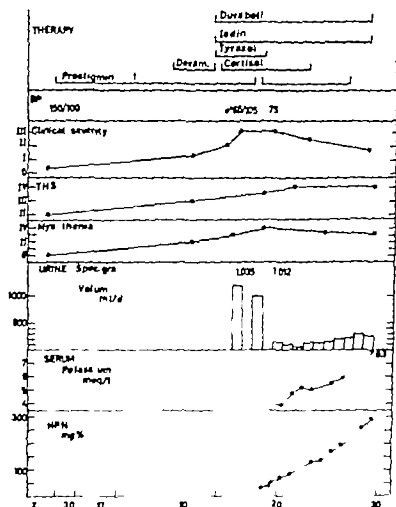


Fig. 1. Graphical presentation of the clinical progression of the thyroid condition, the thyro-hypophysial eye syndrome (THS), the myasthenic symptoms and changes related to the acute renal failure. Thyroid crisis is designated as grade III in clinical severity, malignant exophthalmos as THS grade IV and the severest level of myasthenia as grade IV.

also a few leucocytes which was thought to be due to the continuous catheterization.

During the next day the patient became conscious and understood what was spoken. Some signs of hyperthyroidism seemed to subside. The restlessness disappeared, the transpiration decreased gradually and the temperature went down to 37.5° C. The myasthenic component was difficult to evaluate but seemed to be somewhat better. Mucus appeared in the trachea and pharynx. He could not manage without the respirator although some autonomous movements appeared. The renal failure did, however, continue. The changes in the potassium and NPN are shown in fig. 1. During this time there was an enormous increase in the eye signs. There appeared a very heavy chemosis and excessive swellings around the eyes and a corneal lesion in one eye. The eyes were treated with antibiotic ointments and moist packings. In addition he got a tracheitis and pneumonia, evidently due to the respirator tube.

Laboratory examinations (Oct. 28). Hb 10.2 g/100 ml, RBC 3.2 mill/mm³, haematocrit 35, proteins 5.3, chloride 83 mEq/l, CO_2 16.9 mEq/l, potassium 7.1 mEq/l, sodium 128 mEq/l, calcium 4 mEq/l, phosphate 5.4 mEq/l, WBC 34 700/mm³, NPN 250 mg/100 ml.

At this time it was evident that *if the eyes* were to be treated in some way the only feasible means would have been possibly hypophysectomy or decompressive operation. None of these could have been performed in the state of oliguria. Treatment with heavy doses of corticosteroids did not seem feasible for the same reason. The patient was evidently recovering from the thyroid storm itself but was suffering mainly from the renal failure

and the exophthalmic syndrome. Before any attempt could be made to transport the patient to an artificial kidney in Sweden, he succumbed on Nov 29 in his hyperkalaemia, now 9 0 mEq/l.

Autopsy performed by Dr V Ritama, M. D the hypophysis weighed 0.5 g and appeared unusually hard. The thyroid weighed 22 g and felt firmer than normal. Adrenal glands 14 g, the colour was grey no haemorrhages. In the right lung bronchopneumonic areas. In the trachea and the bronchi some mucus and pus. Persistent thymus was found, the weight being 17 g The liver weighed 1 770 g, and the consistency was somewhat sof ; in the superficial layers (about 1/2 cm) the colour was icteric, in the inner parts unusually pale and the acinar structure seemed to have disappeared. The pancreas weighed 105 g and was somewhat enlarged. The kidneys weighed 220 and 215 g respectively the colour was pale greyish-red. The cortex appeared increased. On histological examination there was found some perivascular oedema in the pons and midbrain. I the liver there were some degenerative changes in the periportal parenchyma, and some inflammatory cells were seen. In the kidneys there were interstitial oedema, lymphoid cells, in the glomeruli slight localised degeneration and the capsule seemed to be thickened with degenerative changes of the capsule epithelium. The tubuli contorti were enlarged and the epithelium degenerated and loosened The same was seen in the loop of Henle I the persistent thymus bout 50 % of the mass consisted of fat and the rest was normal thymus tissue The thyroid contained micronodules, the epithelium was normal. I the skeletal muscles unspecific degeneration was seen. I the ocular muscles there were prominent myodystrophic changes and the subsarcolemmal nuclei were increased in size. Lymphoid cell infiltration was observed. Special fixation in lead acetate of the retrobulbar connective tissue did not reveal any marked increase in metachromatic substance.

Discussion

The patient presented features which may be regarded as typical" of insidiously developing Graves disease The dominating signs were those of the ophthalmoplegic ophthalmopathy of Brain and Turnbull (5) the first symptoms of which evidently were the spells of ache behind the eyes which he experienced already 2 years before the outbreak of the disease. Only in a later stage did hyperthyroidism become manifest and develop within a few weeks into a thyroid storm. The development at this stage likewise illustrates how insidiously the storm may occur Owing to some previous experiences we have been careful with patients who have only slight or moderate signs of hyperthyroidism but evidence of muscular (or neuromuscular?) involvement. As the signs of hypophysial exophthalmic syndrome (swellings, chemosis, injection etc. (25 26)) were only faint, prostigmine treatment was tried on the view that the condition could have been due to myasthenic gravis. During this treatment the muscular activity was improved and later on the interruption of this treatment led to obvious difficulties.

The patient condition during the storm corresponded to that of acute thyrotoxic encephalomyopathy described by Waldenstrom (53) of which the characteristic findings are cerebral phenomena, pseudobulbar palsy and muscular weakness. The pathogenesis of this state is not very well understood. It seems that apart from muscular derangement also neurological lesions might be present. In the present case the high ratio of creatine/creatinine (3 4) (26) suggests the presence of thyrotoxic myopathy Creatinuria is a common finding in this condition and the increase of the ratio creatine/creatinine in plasma may be due to some blocking of the conversion of creatine to creatinine. On the other hand creatinuria is also seen in other muscular conditions.

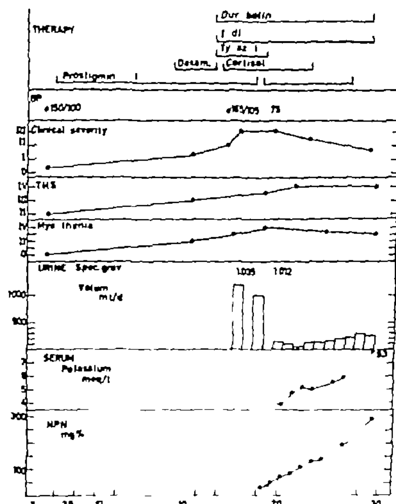


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The muscular involvement in Graves disease may be classified into two groups (37) 1) Cases in which the myopathy is the consequence of the hyperthyroid state a) general muscular weakness without muscular atrophy b) chronic myopathy and c) ophthalmoplegic ophthalmopathy and 2) cases with coexistence of hyperthyroidism and a muscular disorder a) myasthenia gravis and b) familial periodic paralysis It can be questioned whether or not groups 1) a and 1) b are the same condition. In electromyographical studies it has been shown that typical changes are seen in cases without real muscular wasting (16, 43 55) It has also been observed that the incidence of hyperthyroidism is greater in myasthenia gravis than in the normal population (10 40 41)

Much confusion seems to prevail as regards the connections between myasthenia gravis and hyperthyroidism According to some authors (39 40 44) prostigmine should not influence the muscular activity if this is induced by hyperthyroidism accordingly the reaction to prostigmine has been used for a test to differentiate between myasthenia gravis and thyrotoxic myopathy (32 41) On the other hand there are also reports which tell about a positive influence of prostigmine on thyrotoxic myopathy (4 24 30 35 36 47) and some authors have seen a worsening and development of myasthenia gravis during treatment with desiccated thyroid (10 14 39) A positive response has also been seen in cerebral conditions (23) These matters have been recently reviewed by Weickhardt et al (56) and Adams (1) Also the "see-saw' relationship between the two conditions seems not to have been well established (24 32 40 48 56) since some authors have observed such a relationship (2 34 36 45 51) but others not (14, 24 50 58)

In the present case the negative electromyographic findings previous to the crisis seem to have excluded the presence of myasthenia gravis as a separate entity This is not, however entierely unequivocal since Desmet and Monaco (9a) have shown that the electromyographic changes in myasthenia gravis are not uniformly distributed in the skeletal muscles but that the muscles may be involved to varying degrees. Later on prostigmine had however a very marked effect on the muscular condition in the present case. Prostigmine treatment was omitted for a short period on three occasions In the two first instances there was a marked increase in muscular weakness and on the third occasion pseudobulbar and respiratory palsy occurred The very dramatic response to prostigmine on this occasion which made the use of curare necessary in order to install the respirator tube certainly speaks in favour of a prostigmine susceptible muscular condition.

Hence at least some peripheral mechanism was responsible for the respiratory palsy On the other hand, it seems to be impossible to evaluate the role played by purely central mechanisms in acute thyrotoxic encephalomyopathy On histological examinations of the brainstem nothing unusual was observed

The fact remains, however that during the critical state prostigmine had a marked effect on the muscular activity In the autopsy a thymus weighing 17 g was found which contained 50 per cent functioning tissue. One would then be inclined to propose a "latent state of myasthenia gravis" which would be manifested only in certain circumstances. Thyroid crisis involves probably most of

the metabolic events in the organism (20 29 52). Hence, it would not seem too far fetched a supposition that, for instance, acetylcholine could be destroyed at a faster rate in such a condition. This would explain the absence of myasthenic changes a few weeks before the storm and the increased responsiveness to prostigmine later on. Current views on the neuromuscular transmission defect in myasthenia gravis include the possibility of a decreased synthesis or release of acetylcholine or a decreased sensitivity of the endplates (13, 15) and recently an auto-immunisation process has been suggested as the underlying cause in which the thymus could play some role (8, 57). The development of the myasthenic component in the present case would be compatible with an increased destruction of acetylcholine creating an acquired myasthenia which does not exclude the possible presence of an underlying primary defect. Basing their views on observations in one case with a see-saw" relationship MacLean and Wilson (36) reckoned on the contrary that the thyrotoxic myasthenia could be due to rapid destruction or defective production of choline esterase but that in myasthenia gravis a curare like material was produced which was inactivated or not produced when the level of thyroid hormones was increased.

The acute fall in blood pressure was possibly brought bout solely by the respiratory failure per se. On the other hand acute adrenal failure is known to occur sometimes in thyroid crisis (9 29 54). It is also known that the metabolism of corticosteroids is increased in hyperthyroidism 6, 17 22 31 42) along with qualitative alterations in the metabolic pattern of the hormones (17). This would make the pituitary-adrenal axis less able to respond, in accordance with results of corticotropin and methopyrapone tests in hyperthyroidism (7 11 12, 21 38). Also the increased level of ACTH-like material in the blood in hyperthyroidism would suggest a strain in that axis (18).

Renal complications are seldom encountered during the course of hyperthyroidism. Usually there is an increase in the glomerular filtration rate proportional to the increase of the cardiac output (3 19) and also an increase in the creatinine clearance (25). In states with thyroid crisis one of the authors in a previous study (29) was able to observe two patients in whom pre-existing renal failure was greatly enhanced during the crisis. Both apparently recovered from the crisis but soon succumbed of renal failure. It seems thus conceivable that at least with pre-existing renal failure this condition can be greatly aggravated by the thyroid storm. Danowski (9) also mentions that oliguria may occur in thyroid storm. Waldstein et al. (54) on the other hand, did not observe such a course in their patients. The case reported by Sanghvi et al. (46) which recovered spontaneously from the acute thyrotoxic encephalomyopathy with paraplegia and hypokalaemic nephropathy did not develop renal failure. Hypokalaemia seems sometimes to be present in such cases 33). It may also be mentioned that Huth et al. (20a) reported on a case of hyperthyroidism with hypercalcaemia in which renal tubular acidosis was thought to be consequent to nephrocalcinosis.

To the best of our knowledge the occurrence of acute tubular necrosis has not been mentioned specifically in the literature as a complication to thyrotoxic crisis. The present case eport does point to this possibility although it is not known in this case whether the acute fall

in the blood pressure was brought about by the myasthenia or by an acute adrenal failure due to the thyroid crisis

Summary

A report is given of a male patient 31 who died of acute tubular necrosis consequent to encephalomyopathic thyroid crisis Graves disease developed insidiously The history revealed some hyperthyroid features about half a year previously exophthalmic eye symptoms appeared about 3 months before death and the condition developed from an apparently compensated nearly euthyroid clinical state into a full blown encephalomyopathic crisis during a few weeks Concomitantly during the last few weeks there appeared increasing myasthenia and the responsiveness to prostigmine increased during the course of the condition and also the exophthalmic syndrome increased rapidly The possibility is discussed that the metabolic derangement in severe hyperthyroidism and thyroid crisis could induce alterations in the metabolism of acetylcholine which could create a myasthenia gravis-like condition During the height of the crisis there was an acute fall of the blood pressure consequent to which tubular necrosis developed

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Value of the TSH PBI-test in Diagnosis of Hypothyroidism

By

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The response of human thyroid glands to exogenous thyrotropic hormone (TSH) has been used as a measure of thyroid function. In the TSH tests it is assumed that the thyroid responds to TSH in secondary but not in primary hypothyroidism. The thyroid uptake of I^{131} (1, 2, 5, 10, 11, 15, 16) and serum PBI (3, 8) or both (4, 6, 7, 13, 17) has been used in the assessment of the effect of TSH.

The purpose of this study was to check the value of the response of the serum protein-bound iodine (PBI) to TSH in the diagnosis of hypothyroidism.

Methods and material

The TSH test was performed as follows. Blood was withdrawn for PBI analysis after which the patient was given daily i.m. injection of 10 U.S.P. units of TSH (Actyron, Ferring A.B. Malmö) on 3 consecutive days. On the fourth day 24 hours after the last injection of TSH a second blood sample was withdrawn for PBI analysis. The response of the thyroid to TSH was judged from the change in the serum PBI level. The serum PBI was determined by a modification of the Barker method (14).

The series consisted of 147 patients with firm or provisional diagnosis of hypothyroidism (table I). A firm diagnosis required typical symptoms, positive laboratory tests and favourable response to thyroid replacement therapy. The diagnosis of hypopituitarism and the differentiation between primary and secondary hypothyroidism were clearly established by accepting as secondary only those patients with clinical and laboratory evidence of multiple glandular dysfunction of pituitary origin. In the present series the TSH test was thus never used for this differential diagnosis. The group of patients with low thyroid reserve (table I) satisfied the criteria of Jefferies et al. (7) i.e. normal thyroid uptake of I^{131} and normal serum PBI and failure to respond to TSH.

In the group with a provisional diagnosis of hypothyroidism the patients had symptoms suggestive of hypothyroidism, but the usual laboratory tests were equivocal. Some of these patients were receiving thyroid therapy at the time of examination. No patients who had recently been given iodine-containing X-ray media were included, since these substances are known to interfere both with the uptake of I^{131} and the determination of serum PBI. The final decision of whether hypothyroidism was present or not was made on the basis of long follow-up, response to thyroid therapy and reaction to cessation of thyroid therapy whenever suitable. Even so, there were occasional patients in whom there was still

Submitted for publication August 27, 1963

suspected but finally excluded the initial PBI level as well as the response to TSH was normal. In the patients on thyroid medication at the time of examination the initial PBI was either normal or increased and rose following the administration of TSH. The mean rise was somewhat less than normal which was, at least partly due to the fact that in 3 patients TSH produced no increase of the serum PBI These 3 patients had taken about 120, 180 and 240 mg of desiccated thyroid for 3 7 and 17 years, respectively Thyroid medication was then stopped but the patients remained euthyroid (follow-up for 12 months) In these patients the administration of moderate to large doses of desiccated thyroid over a prolonged period had probably produced atrophy of disuse of the thyroid cells which were then unable to respond to even repeated doses of TSH.

Discussion

The response to TSH in well defined primary hypothyroidism is absent or abnormally weak. With the present technique for the TSH-test the largest observed increase in patients with known primary hypothyroidism was 1 5 $\mu g/100$ ml as against 2.1—8.4 $\mu g/100$ ml in the controls. There was thus no overlap between these two groups.

The response was less than normal in hypothyroidism secondary to hypopituitarism. I 2 of 25 patients TSH produced no increase at all. The impairment of the response to TSH in secondary hypothyroidism may be due to atrophy of disuse of the thyroid cells owing to longstanding absence of TSH stimulation. This assumption has been substantiated by Sheehan and Summers (12) finding of extensive fibrosis, lymphocytic infiltration and atrophy or disappearance of thyroid cells in thyroid glands of some patients with anterior pituitary failure. The occasional absence of any response to TSH in patients with secondary hypothyroidism appears too rarely to limit the value of the TSH PBI test in the differential diagnosis between primary and secondary hypothyroidism. It must be admitted, however that careful assessment of the functions of the other endocrine glands, controlled by the anterior pituitary is generally sufficient to differentiate secondary from primary hypothyroidism. Isolated failure of TSH production is too rare to be of any practical clinical significance.

The findings made in the present investigation indicate that the TSH PBI test is an important aid in differentiating euthyroidism from mild and obscure hypothyroidism. The test is most useful when the symptoms and signs of hypothyroidism and the other laboratory tests, including PBI BMR and cholesterol are ambiguous. The test is not always decisive, however As to the failures of the test, it might be convenient to deal first with untreated patients and then with patients who were receiving thyroid medication at the time of the test. In the untreated patient the absence of any response of to TSH is strongly suggestive of hypothyroidism. In untreated patients a response to TSH argues against but does not exclude hypothyroidism. We have observed 2 patients who probably had early hypothyroidism following subtotal thyroidectomy but responded to TSH. Whether such observations should be interpreted as signs of failure of thyrotropin production is not yet clear

In patients taking thyroid preparations a normal response to TSH excludes primary hypothyroidism and the drug can be withdrawn. On the other hand, the

Table I. Results of the TSH — PBI test

Diagnosis	No. of pat.	Serum PBI (μg/100 ml)					
		Pre-TSH		Post TSH		Change	
		Mean	Observed range	Mean	Observed range	Mean (M)	Observed range
Normal subjects	51	5.6	4.7–7.1	11.1	6.2–15.0	5.5	2.1– 8.4
Primary hypothyroidism	30	1.8	0.4–4.3	1.9	0.4–4.9	0.3	−1.0–+1.5
Secondary hypothyroidism (hypopituitarism)	25	3.0	1.0–4.4	7.1	2.4–11.8	4.2	0.2– 9.9
Low thyroid reserve	25	5.6	4.1–7.1	6.0	4.0–7.8	0.3	0.0– 1.4
Hypothyroidism initially suspected							
1) Diagnosis proved							
a) untreated	16	3.2	2.6–4.5	3.5	2.6–6.1	0.3	−0.2–+0.7
b) on thyroid treatment	9	7.8	3.2–12.4	7.9	3.4–12.6	0.1	−0.1–+0.4
2) Diagnosis excluded							
a) untreated	24	3.2	3.7–7.4	10.2	8.6–13.2	5.1	2.3– 9.3
b) on thyroid treatment	18	8.0	4.8–13.2	12.1	6.9–17.5	4.2	1.1– 10.3

some doubt whether hypothyroidism was present or not.

The controls consisted of 16 normal adult volunteers and 35 patients admitted to hospital for diseases unrelated to the thyroid gland such as peptic ulcer, varicose veins, rheumatoid arthritis and psychoneurosis.

Results

The results are summarized in table I. The normal controls showed a mean PBI increase of 5.5 μg/100 ml (range observed 2.1 to 8.4), the group with primary hypothyroidism an increase of only 0.3 μg/100 ml (range — 1.0 to + 1.5) and the group with secondary hypothyroidism an increase of 4.2 μg/100 ml (range 0.2—9.9). As expected, the increase was much smaller in primary than in secondary hypothyroidism. Furthermore, the average response to TSH in patients with secondary hypothyroidism was less than in the controls. This may be due to a possible degeneration of the thyroid cells in long standing hypopituitarism. In 2 patients with hypopituitarism TSH produced no increase of the PBI.

According to definition patients with the low thyroid reserve syndrome do not respond to TSH.

In patients, in whom primary hypothyroidism was initially suspected and later proved, the mean pre-TSH PBI value was 3.2 μg/100 ml with a range of 2.6 to 4.5 μg/100 ml. Though the mean PBI was lower than normal some of the individual values fell within the normal range. TSH produced no significant increase of the PBI. Similar results were obtained in the hypothyroid patients on thyroid medication at the time of investigation, with the exception that the pre-TSH PBI values were either normal or elevated because of the exogenous thyroid hormone supply.

In the untreated patients in the group, in whom hypothyroidism was initially

tients) In the normal subjects the PBI level increased on the average 5.5 μg/100 ml (observed range 2.1—8.4 μg/100 ml) A definite, though somewhat smaller increase of the PBI level was observed in the patients with secondary hypothyroidism (hypopituitarism) In patients with primary hypothyroidism the increase of PBI was always definitely decreased except in two patients with early hypothyroidism after subtotal thyroidectomy In patients, in whom the diagnosis of primary hypothyroidism was excluded after prolonged observation and absence of exposure to thyroid medication, the response to TSH was on the whole of the same magnitude as in the normal subjects though somewhat smaller in patients on thyroid therapy Two patients belonging to this latter category showed no response to TSH despite normal thyroid function.

The findings appear to warrent the conclusion that the TSH PBI-test is most useful for

) Differentiation between true, mild or moderate hypothyroidism and hypothyroidism of questionable character in a euthyroid patient

b) Differentiation between normal thyroid function and true primary hypothyroidism in patients taking thyroid preparations

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absence of response to TSH in patients on thyroid medication will allow a provisional but not firm diagnosis of hypothyroidism since treatment with thyroid preparations over a long period may impair the capacity of the thyroid cells to respond to TSH. It would appear that establishment of a firm diagnosis in such patients requires a few months' withdrawal of the thyroid treatment and subsequent re-evaluation of the patient. It should however be remembered that in such a situation even patients without thyroid disease may experience a short period of transient hypothyroidism.

Previous investigators (2, 3, 4, 6, 15) as well as observations made in the present study show that the response of PBI to TSH is a valuable adjunct in the diagnosis of hypothyroidism which can be used even in patients taking thyroid preparations. As to the best technical procedure for routine use several investigators (1, 2, 5, 9, 10, 11, 15, 16) feel that determination of thyroid uptake of I^{131} before and after a single injection of TSH is sufficient. But such a simple technique assesses only the capacity of the thyroid gland to concentrate iodide. Therefore, as suggested by Bishopric et al. (2) measurement of thyroid uptake of I^{131} after 24 hours or perhaps even longer instead of after 3—6 hours probably improves the accuracy of the test. Since the ability of the thyroid to synthesize and release thyroid hormone must surely be the most important part of thyroid function, it would seem most logical to use a TSH test measuring hormone production, i.e. either the PBI or the PBI^{131} TSH tests based on repeated injections are probably more accurate than those based on single injections since the latter type probably largely indicates the release of stored hormone, while the former types may be considered to be an index of the production of thyroid hormone. When out patients are studied — and most of the patients presenting the actual type of diagnostic problems are out patients — it would seem that a TSH PBI test may be somewhat more practical and time-saving for the patient — and for the laboratory — than a TSH — I^{131}-uptake test, collection of blood samples requiring much less time than an I^{131} test. Furthermore, the TSH PBI test can also be used by practising physicians and small hospitals without isotope laboratories since the blood samples can easily be sent to specialized laboratories for the PBI determination.

Since the TSH preparations used for these tests contain foreign proteins, undesirable though not serious, side effects may be expected (5, 8, 15) e.g. occasional discomfort at the site of injection, occasional pain and tenderness over the thyroid (only in patients who have responded to TSH) and occasional sensations of warmth. No severe allergic reaction has been observed despite the fact that several patients have had repeated TSH tests.

Summary

In the evaluation of the TSH PBI test in the diagnosis of hypothyroidism the serum PBI level was determined before and after a daily injection of 10 U.S.P. units of TSH on 3 consecutive days. The clinical series consisted of 51 normal subjects, 30 patients with known primary hypothyroidism, 25 patients with known secondary hypothyroidism, 25 patients with low thyroid reserve, and 67 patients, in whom hypothyroidism was or had been suspected but not proved at the time of examination (40 patients) or before institution of thyroid medication (27 pa

Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Nine Cases of Hereditary and Non-hereditary Periodic Diseases

By

SVEN E. NILSSON and STEN FLODERUS

In recent years considerable space has been given in the literature to the periodicity of certain symptom complexes (1, 2, 7, 8, 9).

Below a description is given of some personally observed cases, whose symptomatology elucidates interesting aspects of this problem.

Case reports

In the spring 1961 a man (case 1) aged 37 presented himself at the Department of Medicine, Malmö General Hospital, complaining of periodic subcutaneous oedema of varying localization, followed by abdominal pain and migraine. The man's mother (case 2) who had died in 1960 had for several years been observed at the department because of a similar condition, which after the menopause also included a marked tendency to unicaria. The family, which could be traced back 5 generations with the aid of the parish registers, had apparently lived in villages in Scania and none of the members had married Jewish or Armenian partners. The pedigree of the last five generations is given in fig. 1.

The proband's maternal grandfather, who died at 37 years from acute asphyxia probably due to laryngeal oedema, had also had similar symptoms. A brother (case 3) of the proband had on two occasions had laryngeal oedema. An 8-year-old daughter of this brother had recently experienced an initial attack of laryngeal oedema. During the last year an 11-year-old son of the proband had complained of periodic abdominal pain similar in type to that experienced by the proband.

Case 1. Male, born 1924. The patient had felt well until 8 years of age, when he began to have attacks of abdominal pain and vomiting. At about 13 years of age these attacks began to show periodicity with intervals of about 1 month. At 20 years the symptom complex assumed a definite form which afterwards persisted, though the symptoms tended to be more severe. The interval is now 12—14 days, and the attacks last for 2—6 days, usually 3 days. The day before an attack the patient feels in high spirits. The first day of the attack is characterized by local non-itching swelling of varying localization — often in the face, on the backs of the hands, and the lower arm up to the elbow or in the region of the buttocks. Trauma appears to have a localizing effect. For example he had had oedema of the small finger from which capillary blood had been

Submitted for publication August 29, 1963.

Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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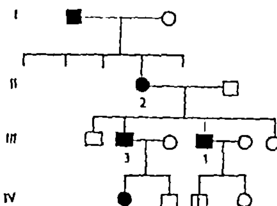


Fig 1 Cases 1 2 and 3. Pedigree of the last four generations. ● Man fest disease [1] Probably affected; ○ Healthy

drawn the day before and in the region of the buttocks after he had been sitting on a hard chair and periorbitally after a long motor tour On the first day of an attack he is very irritable The second day is characterized by abdominal pain often of colicky type abdominal distension repeated vomiting massive heavy stools, followed by fatigue, and sometimes a tendency to faint often making bedrest necessary He suffers from excessive thirst during an attack, he drinks large amounts of soda water The migraine which is usually worst 1—2 days after the abdominal pain — usually has its punctus maximus in the left half of the neck. On the third day the abdominal pain suddenly stops — often in association with violent vomiting and considerable diuresis. During the following few days he has a marked feeling of well being

In Jan 1961 he consulted an ophthalmologist because of headache of persisting type Examination of the ocular fundi revealed a hypertensive retinopathy grade II the blood pressure being 250/150 mm Hg — the increase in blood pressure has, however practically disappeared without any special therapy and no changes are now demonstrable in the fundi. In Sept. 1960 he was submitted to operation because of assumed appendicitis — but the appendix was found to be normal.

Since 1961 the patient has attended the Department of Medicine Malmo General Hospital On two occasions he had an attack while in hospital. The attacks were not accompanied by fever He increased in weight from 68 to 70 kg Laryngoscopic examination revealed on one occasion a swelling of the region of the arytenoid cartilage without clinical symptoms. Laboratory studies during attacks showed leucocytosis with 13,000 white blood cells per mm³ of which 85 % were neutrophils. E.S.R (during attack) max. 30 mm/1 hour Electrophoretic pattern normal except for α_2 0.82 g/100 ml. The A.H.G. was decreased.

Treatment with ergot preparations initially produced a fairly good response and still gives some relief of the headache accompanying the attacks. Prednisone had no demonstrable effect. The results of Mellinkoff's (6) investigation prompted a trial with a low fat diet. This dietary treatment had a favourable effect but after about 3 months the attacks recurred with equal severity despite continued treatment. After the patient had been informed of a possible relationship between the attacks and diet he reported that he had noticed that the severity of the attacks varied somewhat with the amount of food and fluid he consumed When he refrained from eating as soon as peripheral oedema began to appear the abdominal pain did not always occur When the attacks were restrained by therapy however dysphoria occurred, and it took some time before he felt himself again.

The proband reported that apart from the symptoms described he always felt well. Even on heavy exposure he seldom catches cold. On the other hand, he is very susceptible to ant bites, which produce areas of oedema.

Case Female, born 1889 The woman reported an unhappy marriage — she had been divorced in 1938 after 28 years of married life during which she had borne 4 children. As a girl she developed chronic otitis for which she was operated upon at 25 years. Even before she started school she had been troubled by attacks of migraine which have continued at intervals of 1—2 weeks. After few years the attacks of migraine were also accompanied by abdominal pain and severe vomiting The abdominal pain was preceded by subcutaneous oedema, usually of the lower arms and hands, and occasionally of the face In 1950—1952 basal bronchopneumonia recurred. In 1953 the abdominal pain and distension progressed and about 6 l of ascitic fluid was tapped. She was submitted to surgical exploration at which biopsy specimens were removed from nodules

on the omentum. Histological examination revealed necrosis of fatty tissue surrounded by large resorption cells and giant cells of foreign body type as well as fibroblast proliferation and an increase of connective tissue (specimen examined by Professors Wahlgren and Linell). From then on ascitic fluid was tapped at intervals of 2–3 months. Radiotherapy in 1939 produced some improvement. In 1954 and 1956 the patient was troubled by symptoms of roentgenologically verified gastric ulcer. From 1954 on she had a considerable tendency to epistaxis, for which she was repeatedly admitted to the department of Otolaryngology. For several months in 1956 she had intermittent pain of the right leg without any corresponding objective changes. She afterwards also had episodes of pain and sometimes slight swelling of the left knee.

In 1957 she developed progressive visual hallucinations for which she was admitted, in 1960, to mental hospital, where she died that year.

During the years she was under hospital observation moderate changes occurred in the results of laboratory studies. The E.S.R., which was 98 mm/1 hour during the period of ascites, was only 10–20 mm/1 hour during the last years of her life. Electrophoresis usually showed increased α_2 – max. 0.82 g/100 ml – while the highest γ-globulin value noted was 1.04 g/100 ml. The cholesterol, which was determined on few occasions, as largely raised with maximum value of 441 mg/100 ml, but generally between 200 and 300 mg 100 ml. The blood pressure during the observation period was 180–200/100–120 mm Hg and during the last few years roentgen examination had shown slightly enlarged heart with an outline suggestive of hypertension, and ophthalmologic examination had revealed hypertensive retinopathy grade II. The dominating finding at necropsy was the presence of thick fibrin layers in the abdominal cav. The kidneys, liver and spleen were essentially normal. Amyloid could not be demonstrated.

Case 3 Male born 1918. The patient was the son of case 2 and brother of case 5. He had been troubled by otitis since early childhood. He had had radical operation of the right ear in 1932 and of the left in 1936. Since the age of 10 years he had recurrent swellings – usually of the hands but sometimes of other parts of the body such as the feet and face. Abdominal pain and vomiting also occurred, but these symptoms were less pronounced than in case 1. In 1959 and 1960 he was admitted to a department of otology because of life-threatening laryngeal oedema, which was controlled by cortisone treatment. Examination for allergy on that occasion revealed nothing remarkable. During the last 6 months he has been treated with prednisolone, which appears to control the attacks.

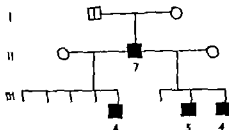


Fig. 2. Cases 4, 5, 6 and 7. Pedigree of the last three generations.

Case 4. Male, born 1931. As in cases 5, 6 and 7 this patient belonged to family described previously by Floderus (4).

One brother (case 5), one half-brother (case 6) and the father (case 7) had died from the same disease. The paternal grandfather died at 57 years of age, according to the death certificate from intussusception. The family originates from the region of Luleå in the north of Sweden.

Since the age of 7 years the patient has had attacks of two types. At intervals of about 1 month he experiences mild attacks of 3–4 days characterized by fatigue, depression, poor appetite and back-pain. A few times a year the attacks are more severe with intense abdominal pain, elevation of the body temperature to 39–40° C, proteinuria (up to 500 mg/100 ml) and E.S.R. of more than 100 mm/1 hour. Laparotomy on 4 occasions during such attacks has revealed distended segments of the small intestine but no fluid in the abdominal cavity. In 1955 the man was admitted to the Medical department, Malmö, but had no attack during the observation period. He had slight proteinuria which, however on one occasion increased to 1 100 mg/100 ml,

and low total serum protein with somewhat increased α_2-globulin. Like an increase of the cholesterol and total lipids, this elevation was ascribed to nephrosis. The fibrinogen was 0.55 g/100 ml. The urinary sediment was normal as was renal function as judged by dilution and concentration tests.

The condition has improved in that the severe attacks are less marked since treatment with a low fat diet was started.

Case 5. Male born 1908. Died 1949. Brother of case 4. The patient had had attacks of abdominal pain since 10 years of age. The attacks started after scarlet fever. The patient had had attacks a few times a year with transverse abdominal pain, high-grade fever, E.S.R. of about 100 mm/1 hour and pronounced proteinuria. During a symptom-free interval 14 months before death the patient had been treated at the Department of Medicine, Karolinska Sjukhuset, Stockholm, because of nephrosis with xanthomatosis. On that occasion, he had had numerous yellow nodules of the skin for about a week. He also had proteinuria (2 000 mg/100 ml). The urinary sediment was normal. The blood cholesterol was 608 and 450 mg/100 ml. In 1949 the N.P.N. increased and the patient died in uraemia. Necropsy showed pronounced amyloid nephrosis.

The descriptions of cases 6 and 7 are incomplete owing to lack of data in the hospital records.

Case 6. Male born 1914. Died 1933. Half brother of cases 4 and 5. Since 7 years of age he had had attacks of abdominal pain. At 8 years he was admitted to Luleå county hospital, where tuberculous peritonitis was assumed. The abdomen was diffusely tender and he had ascites. During the last years of his life he had massive proteinuria.

Case 7. Male, born 1877. Died 1935. Father of cases 4, 5 and 6.

The patient was admitted to the County hospital, Luleå, because of chronic nephritis. He had proteinuria (up to 8,000 mg/100 ml). The urinary sediment showed isolated red blood cells and masses with hyaline and granular casts. E.S.R. 112 and 123 mm/1 hour. In 1961 one of the daughters reported that her father had for several years had periodic abdominal pain of the same type as the sons, but that the pains had decreased at 40—45 years of age. During the last 15 years of his life he had had severe proteinuria.

The father of case 7 had died at 57 years from intussusception, but he had previously been healthy and strong.

Two cases with no known heredity of periodic disease are given below.

Case 8. Male born 1934. Since the age of 8 the patient had had bouts of fever with temperatures up to 39—40° C, swelling of the lymph nodes and diffuse abdominal pain, sometimes associated with diarrhoea. Appendectomy was done in association with an attack of severe abdominal pain. The attacks occurred at different intervals 4—6 times a year and lasted 3—7 days. Histological examination of lymph nodes and the appendix revealed non-specific inflammation without pathological cells. Hormone determinations on urine passed during attacks showed in 1960 and 1961 no increase of etiocholanolone.

During a spell at the Department of Medicine in Malmö in Oct. 1961 the patient had two attacks of the type described above. After the bouts of fever, which lasted 5 days, the patient was hypothermic for a few days — lowest temperature recorded 35.8° C. During the fever the patient had leucocytosis (up to 21 000) due to an increase of neutrophils. One of the attacks was also accompanied by thrombocytosis with a maximum value of 518,000. The number of erythrocytes was unchanged. Biopsy of the lymph node again revealed nothing of interest. Nor did liver biopsy show anything remarkable. Roentgen examination showed enlargement of the liver but the bromsulphalein test was normal. The α_2 globulin increased to a maximum of 0.82 g/100 ml during attacks with a corresponding increase of the haptoglobin to 400 mg/100 ml. Electroencephalography during attacks showed diffuse theta-activity which disappeared after the fever had abated.

Case 9. Female born 1921. (Published previously by Gabinus and Högberg (5).)

No known heredity for periodic disease. Since the age of 19 months the patient had

Table I

	Case 1	2	3	4	5	6	7	8	9
Sex	♂	♀	♂	♂	o	♂	♂	o	♀
Date of birth	1924	1889	1918	1931	1928	1914	1877	1934	1931
Age at onset (yrs)	8	6	10	7	10	7	—	8	4
Age at death (yrs)	—	71	—	—	21	19	55	—	—
Heredity	Dominant			Dominant				0	0
Periodicity	+	+	(+)	(+)	(+)	(+)	(+)	(+)	+
Duration of attack	2–3 days	2–3 days	2–3 days	10–14 days	—	—	—	3–7 days	3 weeks
Fever	0	(+)	0	++	+	+	+	++	++
Abdominal pain	++	++	+	++	+	++	+	(+)	(+)
Vomiting	+	+	+	(+)	+	—	—	—	+
Laparotomy	+	+	0	++	—	+	—	+	—
Ascites	—	++	—	—	—	+	—	—	—
Chest pain	0	+	0	—	—	—	+	—	—
Laryngeal oedema	+	0	++	—	—	—	—	—	—
Joint pain	0	(+)	—	—	—	—	—	+	+
Otitis	—	++	++	—	—	—	—	—	—
Peripheral oedema	+	+	+	—	—	—	—	—	—
Urticaria		+	—	—	—	—	—	—	+
Migraine	++	++	—	—	—	—	—	—	—
Bleeding tendency	—		—	—	—	—	—	—	—
Renal damage	—	—	—	+	++	++	++	—	—
Hypertension			0	0	0	—	—	0	0
Psychic symptoms	—	— —	—	0	—	—	—	—	—
ESR max	30	98	—	115	100	—	123	80	130
Fibrinogen max	—	—	—	0.55	—	—	—		—
Alpha-2 max	0.82	0.82	—	0.50	—	—	—	0.63	—
Cholesterol max	312	441	—	383	608	—	—	—	—
Leucocytes max	13,100	—	—	12,000	9,500	—	—	17,000	35,000
MHG	Decreased		—	—	—		—	—	—
Effect of therapy	Ergotamine ( ) Prednisone 0, low fat diet 0, cholestyramine 0	Rtg ( )	Prednisone ( )	Low fat diet (+)	—	—	—	ACTH (+) antihist. 0, antiglob. 0	ACTH cortisone 0, antihist. 0, Salicyl 0

had attacks of fever with temperatures up to 40° C, vomiting, diarrhoea, urticaria and diffuse muscle and joint pain. At first she had 3—4 attacks a year, later the attacks recurred at shorter intervals, and during the last few years during every other menstruation. The attacks did not occur during the latter part of 2 pregnancies. During attacks the E.S.R. rose to at most 130 mm/1 hour and electrophoresis showed an increase of the α- and β-globulins. In recent years the attacks have responded to ACTH, but prednisone has had no effect.

The most important observations made in case 1—9 are given in table I.

Discussion

The cases described show how difficult it is to make a differential diagnosis in the diffuse group of clinical pictures formerly classified under the heading of "periodic disease".

In cases 1, 2 and 3 the disease was characterized by quite regularly recurring peripheral and visceral oedema, which caused severe abdominal pain and sometimes also life-threatening laryngeal oedema. Migraine was one of the main symptoms. In these cases the disease was evidently inherited through a dominant character and could be traced back through four generations. Though the clinical picture resembles familial Mediterranean fever, it lacks the development of amyloidosis, which appears to be the only finding definitely establishing that diagnosis. It would appear that the cases described fit in best with the diagnosis of hereditary recurrent angioneurotic oedema.

In the family with cases 4, 5, 6 and 7 the attacks of the disease occurred without a definite regular periodicity. All of the members had severe proteinuria. The patient in case 5 had amyloid nephrosis and thereby satisfied the main requirement for a diagnosis of familial Mediterranean fever. The mode of inheritance in this family appears to be dominant, while familial Mediterranean fever according to Sohar et al. (9) is inherited through a recessive character. A further argument against the latter diagnosis is that the disease has so far never been described outside the region of the Mediterranean Sea.

As to cases 8 and 9, the patients had recurrent attacks of fever without known heredity for periodic disease. The symptoms might have been due to some disorder of hormone metabolism similar in type to that occurring in etiocholanolone fever described by Bondy et al. (3) even though we were unable to demonstrate any increased amount of unconjugated etiocholanolone in the urine.

Summary

Nine cases of periodic disease are described. Differential diagnostic problems are briefly discussed.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Depression of Hemolytic Complement Activity of Synovial Fluid in Adult Rheumatoid Arthritis

A Preliminary Report

By

H. Hedberg, Å. Nordén, A. Lundquist and B. Afzelius

The proteins of synovial fluid are considered to originate from plasma proteins (1). The increased amount of protein and the decreased albumin globulin ratio in synovial fluid from arthritic joints in rheumatoid arthritis may be explained by an increased permeability of synovial tissue and of its capillaries, thus enabling larger molecules to enter the joint.

In pilot studies we found that hemolytic complement activity could be demonstrated in synovial fluid from different types of patients, indicating that the complement components, known to be globulins (2), had entered the joint. We then decided to see whether there was any difference in the hemolytic complement activity in synovial fluid between cases of rheumatoid arthritis and control cases. This is the aim of the present investigation.

Material and methods

The patients studied were selected at random among in- and outpatients, all of whom with few exceptions had effusion of the knee joint( ). The clinical material comprised 17 cases of adult, clinically active rheumatoid arthritis (8 females and 9 males, age 40–67 years; mean 55), 2 cases of systemic lupus erythematosus (S.L.E.), both females, aged 57 and 22 years respectively, and 15 control cases (7 females and 8 males, age 34–75 years, mean 53), the diagnosis of which is listed in table I. Eleven cases of rheumatoid arthritis (RA) satisfied 7 criteria for the diagnosis "classical" and 6 cases satisfied 5 criteria for the diagnosis "definite" RA according to the definition of Ropes et al. (9). All cases of RA but one had symmetric involvement of at least metacarpophalangeal or midphalangeal joints of the fingers. No case labelled RA had symptoms of pelvospondylitis. The mean value of the E.S.R. (Westergren) of the cases with RA was 66 mm/h (range 28–115). Five cases were treated with steroids or ACTH. In one case of RA the disease had lasted for 11 months, in 4 cases from 1 to 3 years, and in 12 cases for more than 3 years. One case of RA with a duration of the disease of 2.5 years had a few LE-cells on two occasions without other manifestations of S.L.E.

The sensitized sheep cell (SSC) test performed on whole serum was or had been definitely positive (i.e. agglutination in dilutions $\geq$1 : 64) in 13 out of the 17 cases with RA. The Latex agglutination test (euglobulin frac

Submitted for publication August 30, 1963.

had attacks of fever with temperatures up to 40 C, vomiting diarrhoea urticaria and diffuse muscle and joint pain. At first she had 3—4 attacks a year later the attacks recurred at shorter intervals, and during the last few years during every other menstruation The attacks did not occur during the latter part of 2 pregnancies. During attacks the E.S.R. rose to at most 130 mm/1 hour and electrophoresis showed an increase of the α and β-globulins. In recent years the attacks have responded to ACTH but prednisone has had no effect

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A Preliminary Report

By

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Material and methods

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The sensitized sheep cell (SSC) test performed on whole serum was or had been definitely positive (agglutination in dilutions ≥1:64) in 15 out of the 17 cases with RA. The Latex agglutination test (euglobulin frac

Submitted for publication August 30, 1963.

Table I

Diagnosis	No. of cases	C_S	C_{SF}	Average synovial fluid-serum ratios for									$\frac{C_{SF}}{C_S}$
				Total prot.	Alb.	α_1	α	β	γ	$\alpha+\beta$	Glob.		
Rheumatoid arthritis	17	1,330	2?0	0.67	0.71	0.62	0.45	0.58	0.74	0.54	0.62	0.53	0.19
Systemic lupus erythematosus	2	1,238	110	0.61	0.66	0.60	0.41	0.57	0.67	0.50	0.57	0.47	0.08
Lesions of the menisci	3	1 012	443	0.64	0.70	0.50	0.36	0.57	0.57	0.49	0.51	0.42	0.43
Osteoarthritis	7	1,271	454	0.47	0.51	0.45	0.33	0.35	0.49	0.35	0.39	0.38	0.36
Arthropathia psoriatica	3	1 411	557	0.60	0.65	0.63	0.32	0.47	0.62	0.46	0.61	0.46	0.4?
Pelvospondylitis ossificans	1	1,289	59?	0.74	0.70	0.5	0.45	0.63	1.14	0.55	0.82	0.47	0.46
Uro-polyarthritis	1	1,.14	404	0.53	0.58	0.50	0.33	0.43	0.5.	0.42	0.47	0.44	0.33
Mean		1 214	479	0.55	0.59	0.51	0.34	0.44	0.57	0.42	0.49	0.41	0.39

CH_{50} units in serum (C_S) and in synovial fluid (C_{SF}) respectively

tion) was positive (i.e. agglutination in dilutions ≥ 1 40) in 14 of the cases. These tests were negative in all but one of the control cases. This case exhibited psoriasis, nail changes, sclerosis and destruction of cartilage in one of the sacro-iliac joints and a peripheral arthritis i.a. symmetrically involving metacarpo- mid and distal inter-phalangeal joints of the fingers. The other two cases of psoriasis with peripheral arthritis showed nail changes and involvement of distal interphalangeal joints. One of the control cases had chronic uro-polyarthritis with active prostato-vesiculitis, destruction of bone and cartilage in the sacro-iliac joints and a peripheral arthritis involving wrists and knee joints. One control case had typical pelvospondylitis with peripheral arthritis, three controls had lesions of the menisci and 7 had osteoarthritis.

The two cases with S.L.E. examined during treatment with steroids, showed moderate clinical activity. The E.S.R. values were 102 and 57 mm respectively. Both had a negative SSC titer and one a positive Latex agglutination titer. One patient had LE-cells, a typical rash, pleurisy, carditis, periodic fever and arthritis. The other patient, lacking LE-cells, was characterized by a doubtful rash, nephropathy, carditis, severe anemia, periodic fever and arthritis.

All cases other than three (with lesions of the menisci) had effusion of the knee joint. Only fluids from this joint were studied. Synovial fluid was aspirated and allowed to clot at room temperature for two hours, centrifuged and kept at −50° C. Serum was drawn simultaneously and treated in the same way. No hemolysis was macroscopically apparent in the samples studied.

Hemolytic complement (C′) activity was determined in serum and in synovial fluid in the presence of 0.1 gelatin according to the micro-method described by Wasserman and Levine (10). The details will be given elsewhere. By determining the hemolytic C′ activity (= the no. of $C'H_{50}$ units/ml) of one and the same sample on different occasions the standard error of a single determination was found to be 6.5 for serum and 8.3 for synovial fluid. The protein pattern of serum and of synovial fluid (after treatment with hyaluronidase) was determined by means o

paper electrophoresis according to the method of Laurell et al. (4). In this procedure 10 μl of serum or 20 μl of synovial fluid were applied to the paper. Total protein was determined according to Kjeldahl. Half of the samples had been frozen and thawed once and the remaining samples twice before paper electrophoresis and determination of total protein were performed. Some samples of synovial fluid lacked the β_2-fraction.

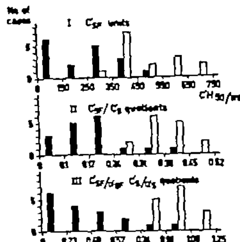


Fig. 1. Hemolytic C'activity in RA (■) and in controls (□) expressed in histogram I as C'H₅₀ units/ml of synovial fluid (C'sf), in II as the quotient (C'sf/C's) between the activity in synovial fluid and that in serum (C's); in III as the quotient between the Y- and X-axis values in fig. 2.

Results

The mean values for hemolytic C'activity expressed as $C'H_{50}$ units/ml of serum (C'_s) and synovial fluid (C'_{sf}) found in different categories of cases are given in table I.

The C' values for the RA cases were slightly but not significantly raised as compared with the controls. The C'_s in the cases of RA was found to vary independently of the E.S.R. and of the SSC and Latex agglutination titers. These results are mainly in agreement with previous reports (5–11).

In table I are included the synovial fluid-serum ratios for total protein and for some protein fractions. For cases of RA and of osteoarthritis these ratios agree closely with the result of a previous study on such cases (6).

The C'_{sf} values in cases of RA were depressed in relation to those of the controls. The difference was statistically significant ($p < 0.001$). In the control cases C'_{sf} and C'_{sf}/C'_s increased with an increase of the protein content of synovial fluid. In the cases of RA there was no such relation. The C'_{sf}/C' ratio was lower in cases of RA as compared with control cases — mean value for RA 0.19 and for controls 0.39 the difference being statistically significant (table I and fig. 1). Two cases of S.L.E. showed ratios of 0.08 and 0.09 respectively.

Comparing the C'_{sf}/C'_s quotient with the ratios for total protein and for protein fractions in the controls (table I) C'_{sf}/C'_s seems to occupy an intermediate position between the synovial fluid-serum quotients of α_1- and α_2-globulins. The C'_{sf}/C'_s agreed most closely with the ratios for total α-globulins in the control cases. This implies that if (in the control cases) the C'activity was expressed as the number of $C'H_{50}$ units/mg of total α-globulins, the synovial C'activity thus expressed (C'_{sf}/α_{sf}) almost equals that of serum (C'_s/α_s). The quotients between the synovial and serum activity thus expressed for cases of RA and controls are represented by histogram III of fig. 1. The cases of RA showed a skew distribution (mean = 0.40). The controls were distributed around a mean of 0.96 (S.D. ± 0.11).

In fig. 2 the ratio C'_{sf}/α_{sf} is plotted against the ratio C'_s/α_s. The regression line for controls is drawn. The correlation

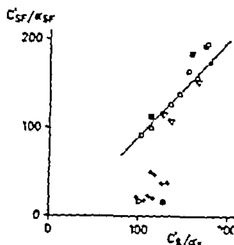


Fig. [illegible] Synovial C' activity in relation to serum activity. α_{sf} and α_s = the concentration (mg/ml) of α-globulins in synovial fluid and in serum respectively.
+ = RA, ○ = S.L.E., [illegible] = osteoarthritis, ⌶ = lesions of the menisci, [illegible] = arthropathia psoriatica, ● = pelvispondylitis ossificans, ■ = uro-polyarthritis.

coefficient for these cases was + 0.83. — If C'_{sf}/C' was plotted against the total protein of synovial fluid the dispersion of controls round the regression line was found to be somewhat larger as compared with the dispersion of controls in fig. 2.

Two cases of S.L.E. both of which had normal C'_s fell within the lower part of the region of RA cases in fig. 2.

Four cases had fresh effusion of the knee joint: two were the cases of S.L.E. just mentioned and two cases belonged to the RA group. Three out of these four cases had depressed C'_{sf}/α_{sf} in relation to C'_s/α_s (fig. 2).

Discussion

The hemolytic C' activity in synovial fluid (C'_{sf}) from cases of clinically active RA was found to be depressed when compared with findings in control cases. A similar observation has recently been published in abstract form by Pekin and Zvaifler (8). In the present investigation the synovial fluid-serum quotient for C' (C'_{sf}/C'_s) was also found to be depressed in RA. In control cases, however, this ratio as well as the number of $C'H_{50}$ units/ml of synovial fluid was found to be increased when the protein content of synovial fluid was increased. Therefore, any comparison based on C'_{sf} or C'_{sf}/C'_s only between control cases and cases of RA seemed to be difficult to perform, if not impossible. By plotting C'_{sf}/C'_s against total protein of synovial fluid or — as is done in fig. 2 — C'_{sf}/α_{sf} against C'_s/α_s a comparison was made possible. The possibility that small amounts of acid glycoproteins may be locally produced and added to the synovial fluid has been discussed by Nettelbladt and Sundblad (7). Such a condition might have influenced the C'_{sf}/α_{sf} quotient.

The depressed hemolytic C' activity in synovial fluid from cases of RA and of S.L.E. may be interpreted as a support for the hypothesis that locally formed antigen-antibody complexes have consumed C'. Against this hypothesis there stands the observation that in this study as well as in previous reports the C' level of serum from cases of RA was essentially normal (5) or even in some cases raised (3, 11). Other mechanisms may therefore have to be considered, for example a local destruction or a decay of C' or inhibition of C' activity. On the other hand little is known about the rate at which C' components are produced or about mechanisms stimulating their formation.

Summary

1. The C' levels of serum and of synovial fluid were determined in 17 cases of RA, 2 cases of S.L.E and in 15 control cases.

2. Methods for expressing the synovial hemolytic C' activity were outlined and discussed.

3. The majority of cases with RA and the two cases of S.L.E. showed a depressed C' activity in synovial fluid in relation to control cases. In three cases out of four with a fresh effusion of the knee joint the C' activity was found to be depressed in the synovial fluid.

Acknowledgement

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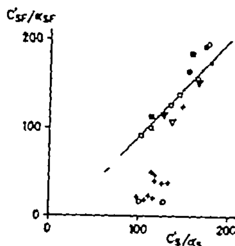


Fig 2 Synovial C activity in relation to serum activity α_{SF} and α_S = the concentration (mg/ml) of α-globulin in synovial fluid and in serum respectively
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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

From the Surgical Department (Head Sverre Thomassen) Nordland County Hospital, Stokmarknes, Norway

Hypercalcemia in Malignant Disease Without Evidence of Bone Destruction

A Case Simulating Acute Hyperparathyroidism

By

Sivert Svane

In recent years there has been a wider recognition of the clinical manifestations associated with hypercalcemia. Predominant features are anorexia, nausea, vomiting, constipation, thirst dehydration, weakness, lassitude and drowsiness. In some cases there is an unusually acute onset and if untreated, progression to coma and death is almost inevitable.

A number of pathological conditions may induce a rise of serum calcium. Hypercalcemia in malignant disease with bone metastases has been well documented. It is most frequently observed in patients afflicted with osteolytic mammary carcinomatosis, appearing in approximately 10—20 % of cases (14 25 27) Serious and even lethal hypercalcemia is also reported in cases of metastatic breast cancer following the administration of estrogens or androgens (12 14 15)

Recently hypercalcemia has been observed in a variety of human cancers without demonstrable bone involvement (6, 18 19) Cancer of the lung (5 6 17 19) and kidney (2 7 18 19) has been the commonest tumor in such cases, but carcinoma of the bladder (7 18) ovary (1 19) uterus (18, 19) uterine cervix (18, 22) vulva (21) pancreas (16) breast (18) liver (hemangioendothelioma) (4) and malignant lymphoma (18, 19) have also been reported. Warwick et al. found that 20 % of cancer patients with hypercalcemia had no clinical or radiological evidence of metastatic bone disease (25) It is remarkable that hypercalcemia simulating hyperparathyroidism with terminal nephropathy is regularly found associated with the anaplastic XV 2 carcinoma of the rabbit (26)

Case report

Female, aged 67 She had always been in good health. Three weeks prior to admission she had painless gross hematuria, followed a few

Submitted for publication September 5, 1963.

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days later by increasing nausea and vomiting. She also complained of vertigo and marked weakness.

On *admission* March 17th 1958 she appeared wasted, weak, nervous and was vomiting constantly. Temperature normal. B.P. 170/70. Pulse 100, regular. Further general examination was normal.

Hb 102 %. Blood urea 86 mg/100 ml. Alkali reserve 48 vols. % CO_2. Prothrombin-proconvertin test 90 %.

There was frank hematuria. Cystoscopy revealed a rather large bladder tumor.

X-ray examination showed no signs of metastases in the lungs, spinal column or pelvis.

Course of illness. The first 2—3 weeks in hospital her general condition was rather poor. There was a temporary rise of blood urea to 137 mg/100 ml and the plasma creatinine concentration was 1.55 mg/100 ml. During parenteral electrolytic fluid therapy there was a certain improvement. The blood urea fell to a normal level. Apart from the first 3—4 days after admission, the daily urinary output was satisfactory. The specific gravity of urine was between 1.014 and 1.024.

The most striking clinical feature was a remarkable weakness combined with constant nausea and anorexia, intermittent vomiting and peculiar mental changes in the form of mild apathy and passivity, slight mental retardation and varying drowsiness. It was hard to explain her clinical picture entirely as manifestations of her apparently localized bladder tumor. The possibility of a hypovitaminosis and Addison's disease was put forward, but the clinical and biochemical investigations excluded these diagnoses.

Her general condition did not permit any operative procedure. There was a rather slow impairment of her symptoms until about May 20th—2 months after admission — when her condition suddenly deteriorated seriously. Her asthenia was alarming. She became increasingly soporous and was constantly vomiting. This new unfavorable course of her disease was also hard to explain. In the morning of May 26th the patient appeared to be near coma. For the first time the determination of the serum calcium was carried out. The her serum calcium was noted to be 19.1 mg/100 ml and the serum phosphorous 1.6 mg/100 ml. (Analyses were made in duplicate. Tests for serum calcium and phosphorous were regularly verified at the University Clinic in Bergen.) Blood urea 68 mg/100 ml. Alkali reserve 61 vols. % CO_2. 50 units of ACTH was administered by intravenous drip. In the evening the same day her serum calcium had fallen to 15.6 mg/100 ml. There was also a noticeable and surprising improvement of her general condition and she appeared to be more conscious.

Reviewing the case history and the biochemical data, there were strong indications of a primary hyperparathyroidism in addition to her bladder cancer. The acute impairment of her condition was ascribed to the development of an acute parathyroid crisis. A surgical exploration of her parathyroid glands was intended to be carried out the following day. On May 27th however she was moribund and became comatose. Serum calcium 17.5 mg/100 ml, phosphorous 2 mg/100 ml, blood urea 60 mg/100 ml, alkali reserve 48 vols. % CO_2. In spite of all therapeutic efforts, including ACTH she died in the evening.

Autopsy. In spite of a systematic and extremely careful dissection of the neck and mediastinum there were no demonstrably enlarged parathyroid glands. A large bladder carcinoma was disclosed. There were multiple metastases in both lungs but no signs of metastases were found elsewhere. The column was split for some distance, but neither macroscopic nor microscopic metastases or osteoporosis could be demonstrated. Numerous small calcified granules were found on the cut surfaces of the kidneys. By squeezing the kidney additional calcified masses appeared. Histological examination revealed moderate nephrocalcinosis. There was a marked cerebral edema. Examination of the heart and suprarenal glands did not reveal any abnormal findings.

Discussion

Our patient suffered from a pronounced hypercalcemia probably secondary to a malignant tumor without evident bone metastases. High calcium

values have been encountered in similar cases. Serum calcium values of 22.5 mg/100 ml are reported (22).

The clinical features were in accordance with those usually associated with the hypercalcemia syndrome.

The combination of a bladder cancer and hypercalcemia is, as mentioned above, reported by other authors (7 18).

There were no signs of enlarged, hyperfunctioning parathyroid glands. This was a surprise since the clinical course and the biochemical investigations strongly suggested a previous undiagnosed primary hyperparathyroidism with a terminal acute exacerbation in the form of the so-called acute hyperparathyroidism. At that time we had recently gained rather depressing experience with two successive cases of this dangerous condition (23 24) and we were convinced that the present case was a third one. Lucas has reported an analogous case, in which urgent parathyroidectomy seemed imperative. On operation however enlargement of the parathyroid glands could not be demonstrated (16). Acute fatal hypercalcemia is also observed during treatment of metastasing mammary cancer with estrogen or androgen (12 15). Withdrawal of the hormonal treatment can be lifesaving.

At the moment, nothing certain is known of the causation of hypercalcemia associated with malignant neoplasms without bone metastases. Three hypotheses have been advanced.

1 Primarily it is an unquestionable fact that a complete or nearly complete removal of the tumor is quickly followed by a return of the serum calcium to normal. The same effect is also observed after X-ray therapy. Hypercalcemia develops again when local or metastatic recurrence becomes apparent. This strongly suggests that the tumors are producing a substance which resembles parathyroid hormone (6). However tumors have been extracted and analyzed for parathyroid hormone activity with negative results (13 21).

2. A second suggestion (19) is that the tumor might produce a substance like vitamin D causing increased calcium absorption from the gastrointestinal tract similar to the defect noted in Boeck's sarcoid (10 11). Howard's objection to this theory is that the speed of restoration to normocalcemia after removal or irradiation of the tumor mass is more like that in a patient after surgical removal of a parathyroid adenoma than that in a patient poisoned with vitamin D or dihydrotachysterol, both of which are cumulative" drugs (13).

3 A third suggestion is the possibility of a functional connection between the tumor and the parathyroid glands. Two cases of malignant disease combined with mild but definite secondary parathyroid hyperplasia are reported by American authors (5 22). In one of these cases there was a striking therapeutic effect of subtotal parathyroidectomy in reducing the serum calcium (22). Primary chief-cell hyperplasia of the parathyroids associated with lung cancer without bone metastases is also reported (17). In all three cases it was suggested that a factor from the neoplasm probably was stimulating the parathyroid glands and acting through them. Hellström reports a patient with metastasing breast cancer and primary chief-cell hyperplasia of the parathyroid glands. The serum calcium was normalized following parathyroidectomy. Hellström regards the case as an occasional coexistence of two different pathological conditions, and does not discuss the possible relationship between them (9).

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Discussion

Our patient suffered from a pronounced hypercalcemia, probably secondary to a malignant tumor without evident bone metastases. High calcium

Summary

A case of marked hypercalcemia associated with a bladder cancer without obvious bone metastases is presented. The diagnosis of hypercalcemia was made shortly before the fatal outcome. The case was misinterpreted as acute hyperparathyroidism, but autopsy did not reveal any signs of enlarged parathyroid glands.

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In most of the reported cases there were no signs of enlargement or hyper function of the parathyroid glands. From a clinical point of view it is highly interesting that the true nature of the case may be misinterpreted as the clinical picture is nearly analogous to that of hyperparathyroidism. This has often resulted in surgical exploration of the neck with negative findings (7 16 19 21)

The *treatment* of hypercalcemia in such cases will to a great extent depend upon the extension and possible scattering of the malignant tumor Where possible, the treatment should be directed towards the basic disease. As mentioned before, an effective therapy of the tumor may lead to a quick restoration to normal serum calcium values with disappearance of the hypercalcemia symptoms (1 2 6 7 13 18 19 21 25)

If a causal treatment is impossible, the management of the more serious cases of hypercalcemia may give rise to serious problems. Although the human calcium tolerance will vary greatly a value of 17 mg/100 ml must be regarded as critical (3) The establishement and maintenance of fluid and electrolytic balance is imperative. These measures may be sufficient in the milder cases. There is no prevailing agreement as to the effect and value of the remedies used in reducing the blood calcium. By parenteral supply of hydrocortisone and prednisone, at least a temporary reduction of the calcium values seems to be attainable (14 15 22 25) In the more serious cases of hypercalcemia associated with malignant disease, Warwick et al (25) recommend at least 3 000 ml of intravenous fluids daily and 100 mg of hydrocortisone in each liter of fluid When vomiting ceases, the patient is placed on prednisone 20 to 30 mg daily and then when the patient's condition improves sufficiently the prednisone is gradually withdrawn The authors stress that they have little experience with the use of chelating agents such as sodium ethylenediamine tetraacetate (EDTA) (8 20) However with the above mentioned measures calcium values returned to normal levels in half the patients treated and remissions of as long as 18 months were obtained Most of these patients were able to return to a comfortable and useful life (25)

Preparations containing estrogen or androgen should be rejected

Regarding the treatment of our patient, it must be taken into consideration that her grave hypercalcemia was disclosed a few days prior to death when the patient nearly was in extremis. Hoping to improve her condition sufficiently to carry out a parathyroidectomy ACTH was given intravenously A certain effect of ACTH cannot be excluded as there was a concomitant drop in serum calcium from 19 1 to 15 6 mg/100 ml with simultaneous improvement of her general condition It is, however impossible to estimate her situation properly since her advanced malignant disorder made all therapeutic measures hopeless. With our present knowledge we would prefer infusions of hydrocortisone and prednisone in a case like this. At that time (1958) however there were only scanty reports on the effect of these agents.

As a conclusion it seems justifiable to emphasize the two noteworthy clinical observations from the present case

1 Hypercalcemia may appear as a complication to malignant disease even without obvious bone metastases.

2 The possibility of malignant disease must be considered in any case of hypercalcemia.

Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Studies in Sarcoidosis

I. Serum Proteins

By

Rudolf Norberg

The changes in the serum proteins in sarcoidosis have been described in a number of previous works. As early as 1935 Salvesen pointed out the occurrence of hyperproteinaemia (15). In 1942 Fisher and Davies (3) showed by electrophoresis that in 6 out of 12 patients there was a reduced albumin content and an increased γ-globulin value. In some cases there was an increase also in α_2- and β-globulins. Similar results were published later among others by Seibert and Nelson (16), Seibert et al. (17), McCuiston (11), Sunderman and Sunderman (21) and Fröhlich (4). On the other hand Mather et al. (12) in a study of 93 cases of sarcoidosis found *only 10 patients with hyperglobulinaemia*.

The diagnostic significance of changes in the serum proteins is difficult to evaluate in earlier works, since, as a rule, the number of cases dealt with are few and frequently clinical data are lacking.

The aim of the present investigation was to study the serum protein pattern on a larger collection of patients with sarcoidosis and to correlate the results obtained with the different stages of the disease. Moreover, in a smaller number of cases the relationship of the total protein to posture has been studied.

Material

Eighty two patients with sarcoidosis of the hilar lymph nodes and/or the lung parenchyma were investigated. Patients — in all five — with signs of other concurrent disease had been excluded from the material. All the patients, except four cases which were observed at Karolinska sjukhuset, were under control at the Department of Pulmonary Diseases of S:t Görans sjukhus. In 11 cases the diagnosis was supported by biopsy from lymph node, either from the supraclavicular fossa or from the mediastinum. The biopsy material and repeated gastric lavages were investigated, by means of guinea-pig tests and cultures, for the presence of tubercle bacilli. The results were negative in all cases. In addition to the routine blood and urine analyses, bilirubin, alkaline phosphatases, GOT, GPT, cholesterol, serum sodium, potassium and calcium, non-protein nitro-

Submitted for publication September 12, 1963

Table II. Group II. Patients with disseminated parenchymal pulmonary lesions

Case no.	Sex	Age (yrs)	Time after 1st pos. X-ray	Time after last normal X-ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %) Albumin	Globulin α_1	α_2	β	γ	Remarks
27	♀	23	1	<1	8.4	64.5	3.2	6.6	12.4	13.3	—
28	♂	29	1	<1	8.4	61.4	3.2	8.4	10.1	14.9	Scar swellings
29	♀	34	1	<4	9.9	56.9	3.7	9.2	9.4	20.8	—
30	♀	33	1	<4	7.8	64.2	3.1	9.9	8.0	13.8	—
31	♀	38	1	<2	8.8	55.6	7.3	7.3	10.2	19.6	—
32	♀	22	1	<1	8.2	53.3	5.0	8.5	12.6	20.6	—
33	♀	45	1	<5	7.6	63.4	5.6	8.3	8.7	14.0	—
34	♀	45	1	<3	8.3	65.3	5.8	7.8	8.9	12.2	—
35	♀	43	1	<13	7.6	60.7	5.0	7.4	11.1	15.8	—
36	♀	34	1	<5	7.4	56.3	5.1	6.1	9.7	22.8	Iritis
37	[illegible]	48	2	<3	8.2	58.4	6.2	8.0	11.8	15.6	—
38	♂	37	2 mos.		8.9	51.3	4.9	10.5	11.2	22.1	—
39	♂	30	2	<6	7.6	59.5	6.9	7.8	12.7	13.1	—
40	♀	31	2		7.7	56.6	3.8	11.4	13.1	15.1	—
41	♂	31	3	<7	9.6	56.8	5.9	6.7	8.3	22.3	—
42	♀	33	3	1	7.0	65.1	4.3	5.3	9.4	15.9	Joint symptoms
43	♀	33	6	<3	7.9	64.2	3.9	6.4	11.4	14.1	—
44	♂	27	9	?	7.9	58.4	6.2	8.2	10.9	15.3	—
45	♂	28	10	5	8.2	60.6	6.1	7.0	10.5	15.8	—
46	♂	44	1	<8	7.7	53.0	2.5	6.8	9.4	28.5	—
47	♀	47	12	<5	8.2	56.5	4.4	8.1	13.5	17.5	—
48	[illegible]	62	18	<5	6.0	53.3	5.7	9.5	9.2	22.3	
49	♀	23	18	<3	7.7	63.0	3.5	9.1	9.4	15.0	—
50	♀	31	18	<3	7.2	56.7	5.4	8.9	12.7	16.3	—
51	[illegible]	49	2	<6	8.2	55.2	6.0	7.1	10.8	20.9	—
52	♂	36	2	3	7.9	52.3	3.8	10.0	12.3	19.6	—
53	♂	34	2	<3	8.0	63.3	4.3	6.3	10.8	15.0	—
54	♂	19	2	<4	6.0	56.8	4.5	6.2	14.0	16.5	Kidney insuff. Serum cholest. 300 mg/100 ml
55		33	3 years	4	7.8	63.7	3.5	5.5	12.3	15.0	—
56	♂	40	3	<7	8.0	62.3	3.9	7.4	7.6	18.8	—
57		31	3		8.3	60.0	5.5	6.2	10.6	17.7	—
58		46	7	9	8.1	60.1	5.7	7.6	10.2	16.4	—
59	[illegible]	37	7	10	7.8	58.7	5.8	7.3	11.2	17.0	—
60	♂	33	8	?	8.1	60.6	5.8	5.7	11.7	16.2	—

work.) In the remaining patients the disease was discovered after routine roentgenogram of the lungs.

At the time of investigation 8 patients still had symptoms. In all the cases the tests for liver and kidney function, and for blood electrolytes were normal.

The group where the disease was progressive consisted of 10 patients. (Indicated by fat types in the table.)

Group II comprised 34 patients (15 men and 19 women) with disseminated parenchymal pulmonary lesions with or without hilar gland involvement, but without roent

Table I Group I Patients with bilateral hilar lymphadenopathy

Case	Sex	Age (yrs)	Time after 1st pos X-ray		Time after last normal X ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %)					Remarks
							Albumin	Globulins				
								α	α	β	γ	
1	♀	2	1	day	<1	7.7	53.3	6.0	8.5	9.7	22.7	Joint symptoms
2	♂	35	1		<1	8.1	51.7	6.5	9.4	13.2	19.2	Joint symptoms
3	♂	33	1		<3	7.4	62.0	4.7	8.1	11.0	14.2	AV-blocking
4	♀	31	1		<1	8.5	64.0	3.8	7.5	9.3	15.4	Scar swellings
5	♂	41	2	weeks	<3	8.6	59.2	4.8	10.2	8.0	17.8	–
6	♂	31	2		<1	7.4	60.6	3.3	8.9	11.5	15.5	–
7	♀	55	2		<1	8.1	54.6	5.4	11.0	12.5	16.5	Scar swellings
8	♀	24	1		<1½	8.4	57.6	4.8	5.5	14.0	18.1	General lymphatic enlargement
9	♀	24	1		<6	8.9	57.9	6.7	10.3	9.1	16.0	–
10	♀	43	1		<3½	8.4	61.5	5.8	7.1	7.8	17.8	–
11	♀	40	1		<1	8.2	60.3	6.3	8.2	10.6	14.6	Scar swellings
12	♂	44	1		<1	8.2	53.[illegible]	5.7	7.6	14.5	19.0	Joint symptoms
13	♀	26	1		<1	8.1	55.4	7.6	11.1	10.4	15.5	–
14	♂	21	1½	mos.	<1½	8.7	60.2	4.5	8.0	8.5	18.8	–
15	♂	22	2		<1½	7.8	59.8	5.1	6.7	13.7	14.7	–
16	♀	20	[illegible]		<1½	8.1	56.9	5.9	7.8	9.9	19.5	–
17	♀	24	2		<1	8.4	64.0	4.9	6.8	10.5	14.0	–
18	♂	31	3		<1	7.8	61.1	4.8	8.8	9.0	16.3	–
19	♂	26	5		1	8.0	61.6	3.5	6.1	8.8	20.0	–
20	♂	27	5		2	7.5	66.5	5.5	5.5	8.0	14.5	–
21	♂	29	6		<2	8.1	63.3	2.6	6.7	11.1	16.3	–
22	♂	27	1		4	7.9	62.5	4.8	7.4	10.9	14.4	–
23	♀	42	1½		3	8.2	57.8	4.0	9.0	[illegible]	17.0	–
24	♀	37	2	years	6	7.8	60.5	3.8	6.4	9.1	20.2	–
25	♀	44	4		10	7.2	61.8	6.6	7.3	11.7	12.6	–
26	♂	39	8		<10	7.2	62.9	6.8	7.7	8.3	14.3	–

gen and in many cases, the exogenic creatinine clearance were determined

The material was divided into different groups according to the roentgenological changes. The division into groups corresponded to the three stages of the clinical course of intrathoracic sarcoidosis Enlargement of hilar lymph nodes (BHL) more or less pronounced disseminated pulmonary lesions with or without hilar lymphadenopathy and a chronic fibrotic stage (10 23 24) In each group those patients, who showed signs of current progress either roentgenologically or clinically were placed in a subgroup. This classification was made by the head and the staff of the department without their knowledge of the results of the protein analyses. The author did not take part in that work.

Group I consisted of 26 patients (13 men and 13 women) with sarcoidosis of the hilar lymph nodes (BHL) but without any parenchymal lesions of the lungs roentgenologically (table I) Ten patients had their lungs radiographed on account of symptoms such as arthralgia, scar swellings and eye trouble. (Cases with concurrent erythema nodosum will be described in a subsequent

Table II. Group II. Patients with disseminated parenchymal pulmonary lesions

Case no.	Sex	Age (yrs)	Time after 1st pos. X-ray	Time after last normal X-ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %)					Remarks
						Albumin	Globulins				
							α_1	α_2	β	γ	
27	♀	23	1	<1	8.4	64.5	3.2	6.6	12.4	13.3	–
28	♂	29	1	<1	8.4	61.4	5.2	8.4	10.1	14.9	Scar swellings
29	♀	34	1	<4	9.0	56.9	3.7	9.2	9.4	20.8	–
30	♀	33	1	<4	7.8	64.2	3.1	9.9	9.0	13.8	–
31	♀	38	1	<2	8.8	55.6	7.3	7.3	10.2	19.6	–
32	♀	22	1	<1	8.2	53.3	5.0	8.5	12.6	20.6	–
33	♀	45	1	<5	7.6	63.4	5.6	8.3	8.7	14.0	–
34	♀	45	1	<3	8.3	65.3	5.8	7.8	8.9	12.2	–
35	♀	43	1	<13	7.6	60.7	5.0	7.4	11.1	15.8	–
36	♀	34	1	<5	7.4	56.3	5.1	6.1	9.7	22.8	Iritis
37	♂	48	2	<3	8.2	58.4	6.2	8.0	11.8	15.6	–
38	[illegible]	37	2	?	8.9	51.3	4.9	10.5	11.2	22.1	–
39	[illegible]	30	2 mos.	<6	7.8	59.5	6.9	7.8	12.7	13.1	–
40	♀	31	2	?	7.7	56.6	3.8	11.4	13.1	15.1	
41	♂	31	3	<7	9.6	56.8	5.9	8.7	8.3	22.3	–
42	♀	33	3	<1	7.0	65.1	4.3	5.3	9.4	15.9	Joint symptoms
43	♀	55	6	<3	7.9	64.2	3.9	6.4	11.4	14.1	–
44	♂	27	9	?	7.9	59.4	6.2	8.2	10.9	15.3	–
45	[illegible]	28	10	<3	8.2	60.6	6.1	7.0	10.5	15.8	–
46	♂	44	12	<8	7.7	53.0	2.3	6.8	9.4	28.5	–
47	♀	47	12	<5	8.2	56.5	4.4	8.1	13.5	17.5	–
48	♀	62	18	<5	8.0	53.3	5.7	9.5	9.2	22.3	–
49	♀	23	18	<3	7.7	63.0	5.5	9.1	8.4	15.0	–
50	♀	31	18	<3	7.2	56.7	5.4	8.9	12.7	16.3	–
51	♀	49	2	<6	8.2	55.2	6.0	7.1	10.8	20.9	–
52	♂	36	2	<3	7.9	52.3	5.8	10.0	12.5	19.6	–
53	[illegible]	34	2	<3	8.0	65.3	4.3	6.3	10.8	15.0	–
54	♂	19	2	<4	8.0	56.8	4.5	8.2	14.0	16.5	Kidney insuff. Serum cholest. 300 mg/100 ml
			years								
55	[illegible]	33	3	<4	7.8	63.7	3.5	5.5	12.5	15.0	–
56	[illegible]	40	3	<7	8.0	62.3	3.9	7.4	7.6	18.8	–
57	♂	31	5		8.5	60.0	5.5	8.2	10.6	17.7	–
58	♀	46	7	9	8.1	60.1	5.7	7.6	10.2	16.4	–
59	♀	37	7	<10	7.8	58.7	5.8	7.3	11.2	17.0	–
60	♂	35	8	?	8.1	60.6	3.8	5.7	11.7	16.2	–

work.) In the remaining patients the disease was discovered after routine roentgenogram of the lungs.

At the time of investigation 8 patients still had symptoms. In all the cases the tests for liver and kidney function, and for blood electrolytes were normal.

The group where the disease was progressive consisted of 10 patients. (Indicated by fat types in the table.)

Group II comprised 34 patients (15 men and 19 women) with disseminated parenchymal pulmonary lesions with or without hilar gland involvement, but without roent

Table III Group III Patients with fibrotic pulmonary lesions

Case no.	Sex	Age (yrs)	Time after 1st pos. X-ray (yrs)	Time after last normal X-ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %)					Remarks
						Albumin	Globulins				
							α_1	α_2	β	γ	
61	♀	58	1	5	8.9	50.8	3.4	8.4	9.3	28.1	Dyspnoea Periods of hypercalcaemia
62	♀	40	4	7	8.1	54.0	3.8	8.4	12.6	19.2	Dyspnoea Periods of renal insuff Hypercholesterolaemia
63	♂	55	4	6	7.8	55.4	6.0	7.0	13.5	18.1	Dyspnoea Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia
64	♂	34	4	10	7.5	67.2	5.4	5.0	8.7	13.7	Dyspnoea Spontanous pneumothorax
65	♀	55	5	?	8.0	61.5	3.8	8.3	9.0	13.4	Dyspnoea
66	♀	36	5	?	8.2	55.0	5.0	9.6	9.3	21.1	Dyspnoea
67	♂	44	5	12	9.4	48.0	4.3	7.5	11.2	29.0	Dyspnoea Periods of hypercalcaemia Periods of renal insuff
68	♂	42	6	12	8.3	58.0	5.7	9.0	10.1	17.2	–
69	♀	38	6	?	8.[illegible]	54.8	3.9	7.4	8.6	25.3	Dyspnoea
70	♀	65	6	10	8.7	51.5	6.8	6.5	13.8	14	Dyspnoea Hypercholesterolaemia
71	♂	44	7	11	8.1	49.2	7.1	8.5	8.9	26.3	–
72	♂	54	7	8	8.0	56.0	7.8	7.6	12.7	15.9	Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia
73	♂	47	7	8	7.1	51.1	5.4	10.0	15.4	18.1	Dyspnoea Periods of hypercalcaemia Periods of renal insuff Hypercholesterolaemia
74	♂	29	8	10	9.2	41.3	5.9	9.2	12.3	31.3	Dyspnoea Spontanous pneumothorax Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia Death from cardiac failure
75	♂	48	9	10	7.9	44.6	6.9	10.5	15.7	22.3	Dyspnoea Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia

Table III Group III (cont.)

Case no.	Sex	Age (yrs)	Time after 1st pos. X-ray (yrs)	Time after last normal X-ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %)					Remarks
						Albumin	Globulin				
							α_1	α_2	β	γ	
76	♀	48	8	11	9.4	55.0	5.4	8.0	7.2	**24.4**	Dyspnoea
77	♀	46	12	?	7.7	59.0	3.6	6.0	10.1	**21.3**	—
78	♀	43	12	21	**8.2**	**45.0**	7.0	9.4	10.0	**20.6**	Dyspnoea Periods of hypercalcaemia Periods of renal insuff.
79	♀	44	14	16	**8.4**	**54.8**	5.5	7.4	9.1	**23.7**	Dyspnoea Spontaneous pneumothorax Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia Death from cardiac failure
80	♀	63	15	?	8.2	59.4	6.7	6.8	9.7	17.4	Dyspnoea Periods of hypercalcaemia Periods of renal insuff
81	♀	31	23	?	**8.4**	**54.0**	4.6	8.1	**13.8**	19.5	Hypercholesterolaemia
82	♀	53	24	?	**8.7**	56.9	**8.3**	6.4	9.3	19.1	—

genological signs of fibrosis (diss. parenchym.) (table II). In 12 of the patients the disease was discovered after the lungs were radiographed on account of subjective symptoms — mainly the same as in group I — whereas in the remaining patients it was detected after a routine roentgenogram of the lungs. When the investigation was carried out 3 of the patients still had symptoms. In all cases except in case No. 54 the liver and kidney function tests yielded normal results (table II). Blood electrolytes were normal in all patients.

The group where the disease was progressive consisted of 12 patients. (Indicated by fat types in the table.)

Group III consisted of 22 patients (11 men and 11 women). All of them showed fibrotic lesions of the lungs (table III). The observation period for patient No. 61 was only 6 months, for the others between 4 and 24 years. The majority had dyspneic discomfort, two patients had had repeated spontaneous pneumothoraces, 8 revealed periodic hypercalcaemia (> 5.5 mEq/l) with more or less pathological kidney function tests. Hypercholesterolaemia (> 300 mg/100 ml) occurred in 8 patients. No patient was on steroids. Later two patients died of cardiac failure (cor pulmonale).

The group where the disease was progressive consisted of 11 patients. (Indicated by fat types in the table.)

The control material consisted of hospital personnel (12 men and 13 women) aged 20—60 years. Their chest roentgenograms were without remark and none had any clinical signs of disease. Their E. S. R. and haemoglobin values were also normal.

Methods

Blood samples were taken either during the patients hospital stay or usually during a visit to the outpatients department. The

Table III Group III Patients with fibrotic pulmonary lesions

Case no.	Sex	Age (yrs)	Time after 1st pos. X-ray (yrs)	Time after last normal X-ray (yrs)	Total protein (g/100 ml)	Electrophoresis (rel. conc. in %)					Remarks
						Albumin	Globulins				
							α	α_2	β	γ	
61	♀	58	/ₜ	5	8.9	50.8	3.4	8.4	9.3	28.1	Dyspnoea Periods of hypercalcaemia
62	♀	40	4	7	8.1	54.0	5.8	8 4	12.6	19.2	Dyspnoea Periods of renal insuff Hypercholesterolaemia
63	♂	35	4	6	7.8	55 4	6.0	7.0	13.5	18.1	Dyspnoea Periods of hypercalcaemia Periods of renal insuff Hypercholesterolaemia
64	○	34	4	10	7.5	67.2	5.4	5.0	8.7	13.7	Dyspnoea Spontanous pneumothorax
65	♀	55	5	?	8.0	61.5	5.8	8.3	9.0	15.4	Dyspnoea
66	♀	36	5	?	8.2	55.0	5.0	9.6	9.3	21.1	Dyspnoea
67	○	44	5	12	9 4	48.0	4.3	7.5	11.2	29.0	Dyspnoea Periods of hypercalcaemia Periods of renal insuff
68	○	42	6	12	8.3	58.0	5.7	9.0	10.1	17.2	–
69	♀	38	6		8.2	54.8	3.9	7 4	8.6	.3.3	Dyspnoea
70	♀	65	6	10	8.7	51.5	6.8	6.5	13.8	21 4	Dyspnoea Hypercholesterolaemia
71	♂	44	7	11	8.1	49..	7 1	8.5	8.9	26.3	–
72	○	54	7	8	8.0	56.0	7.8	7.6	12.7	15.9	Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia
73	♂	47	7	8	7 1	51 1	5.4	10.0	15.4	18.1	Dyspnoea Periods of hypercalcaemia Periods of renal insuff Hypercholesterolaemia
74	○	29	8	10	9.2	41.3	5.9	9.2	12.3	31.3	Dyspnoea Spontanous pneumothorax Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia Death from cardiac failure
75	♂	48	9	10	7.9	44.6	6.9	10.5	15.7	2 .3	Dyspnoea Periods of hypercalcaemia Periods of renal insuff. Hypercholesterolaemia

The mean value for the *total protein* in serum was increased in all the groups where the disease was progressive, but it was either normal or only slightly increased in the groups with stationary lesions.

The mean value for *albumin* was lowered in all the groups.

α_1-*globulin* was normal.

The mean value for α_2-*globulin* was increased in all groups.

The mean value for β-*globulin* was increased in the groups with progressive BHL-cases, stationary parenchymal and stationary fibrotic patients.

The mean value for γ-*globulin* was increased in the groups with progressive disseminated parenchymal lesions and progressive fibrotic lesions, especially in the latter group. For all other groups the mean value of γ-globulin was normal.

On comparing the different progressive groups with the corresponding groups with stationary lesions the mean value for albumin was found to be lower in all the progressive groups. Otherwise there was no statistically significant difference between the values, except as regards γ-globulins which were higher in the fibrotic group with progressive cases than in the corresponding group with stationary lesions.

Within the respective groups there was no definite correlation between the electrophoretic pattern and the duration of the disease.

Effect of posture

The morning values for total protein were the same in the sarcoidosis and the control groups. When comparisons were made after the patients had been up for some time the total protein was found to be elevated in both groups, but the increase was statistically higher in the

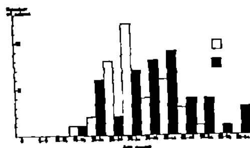


Fig. 1. Age and sex distribution in the entire material.

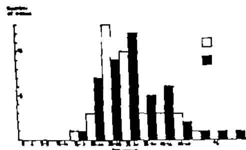


Fig. 2. Age and sex distribution at the onset of the disease.

sarcoidosis group. The relative electrophoretic values for the groups were unaffected by the patient's posture.

Discussion

Earlier autopsy and biopsy investigations have shown that the division into groups based on roentgenograms fairly well agrees with the corresponding pathological lesions (9, 14).

In the BHL-group control roentgenograms were usually performed every second or third month during the first year after the disease had been discovered, but it is of course impossible even in this group to exclude periods with parenchymal lesions. Moreover lung biopsy has shown the presence of sarcoid granulomas in the

Table II Experimental errors

	Total protein (g/100 ml)	Albumin (%)	Globulins (%)			
			α	α_2	β	γ
Normal sera (n = 25)						
Mean value	7 74	60.8	3.8	6.9	10.6	15.9
S	0.08	1 4	0.6	0.5	0.7	1.3
%	1.0	2.3	10.3	7.2	6.6	8.2
Pathological sera (n = 25)						
Mean value	9.37	44.2	7 1	8.5	[illegible]	26.3
S	0.13	3.1	0.6	0.9	0.9	2.5
	1 4	7.0	8.5	10 6	7.0	9.5

$$S = \sqrt{\frac{\Sigma(d\ )}{2n}}$$

serum was centrifuged within two hours and stored at − 18° C until the analysis was made

The *total protein* in serum was determined by a biuret method standardized by Kjeldahl determination of the protein nitrogen content. The usual factor 6 25 was used to convert nitrogen value into protein.

Paper electrophoresis was carried out in a barbiturate buffer pH 8 6, and ionic strength 0 1 The filter paper strips (LKB 140 g/m²) were stained with amido-black and read in an analytrol scanner (Spinco Model RB) The strips were cut according to the diagram obtained the dye was eluted and the concentration of the eluted dye was measured in a photometer at 630 mμ (5)

The experimental errors were calculated for 25 normal and 25 pathological sera (table IV)

Influence of posture Total protein was determined and electrophoresis done in two serum samples the first of which was taken after a night s rest and before the patient got up and the second after the patient had been in supine position for six hours.

The statistical calculations were made according to conventional methods (19) Significance of differences between groups was tested by the t test. The degree of probability was designated as follows

P ₹ 0 05 probably significant (x)
P ₹ 0 01 significant (xx)
P ₹ 0 001 highly significant (xxx)

Individual values outside the normal ranges (the mean value ± 2 SD are indicated by Italics in the tables.

Results

The age and sex distribution of the entire material is shown in fig 1 Fig 2 shows these distributions at the onset of the disease. The sex distribution and the patient s age when the disease was discovered are the same as those usually found in sarcoidosis.

Individual values for total protein and for paper electrophoresis in the sarcoidosis groups are given in tables I—III and in fig 3 The mean values for the control material and various sarcoidosis groups are given in table V

The significance of the difference between the groups is given in table VI

With regard to the results obtained the following facts should be noted particularly

Table V Values for total protein and paper electrophoresis of serum. Mean value ± SE of mean and (below) SD are given

Clinical group	Total no. of cases	Total protein (g/100 ml)	Albumin (%)	Globulins (%)			
				α_1	α_2	β	γ
Controls	25	7.5 ± 0.1 0.4	63.4 ± 0.7 3.3	4.8 ± 0.2 1.2	6.5 ± 0.2 0.9	9.6 ± 0.2 1.2	15.7 ± 0.5 2.5
BHL							
Whole group	26	8.0 ± 0.1 0.4	59.6 ± 0.7 3.7	5.2 ± 0.2 1.2	8.0 ± 0.3 1.6	10.5 ± 0.4 1.9	16.7 ± 0.5 2.4
Progressive cases	10	8.2 ± 0.1 0.3	56.7 ± 1.3 3.9	5.6 ± 0.3 1.1	8.8 ± 0.5 1.6	11.6 ± 0.7 2.3	17.4 ± 0.8 2.5
Stationary cases.	16	7.9 ± 0.1 0.4	61.4 ± 0.5 2.1	4.9 ± 0.3 1.2	7.5 ± 0.3 1.4	9.8 ± 0.3 1.3	16.4 ± 0.6 2.3
Diss. parenchyma							
Whole group	34	8.0 ± 0.1 0.5	59.1 ± 0.7 4.0	5.0 ± 0.2 1.2	7.8 ± 0.3 1.5	10.8 ± 0.3 1.6	17.4 ± 0.6 3.5
Progressive cases	12	8.3 ± 0.2 0.6	57.6 ± 1.0 3.5	4.9 ± 0.4 1.4	8.5 ± 0.4 1.5	10.5 ± 0.5 1.7	19.2 ± 1.2 4.2
Stationary cases	22	7.9 ± 0.1 0.4	60.3 ± 0.8 3.9	5.1 ± 0.2 1.1	7.4 ± 0.3 1.3	11.0 ± 0.3 1.6	16.3 ± 0.6 2.8
Fibrosis							
Whole group	22	8.3 ± 0.1 0.6	53.8 ± 1.3 5.9	5.7 ± 0.3 1.3	8.0 ± 0.3 1.4	10.9 ± 0.5 2.4	21.5 ± 1.0 5.0
Progressive cases	11	8.7 ± 0.2 0.6	50.0 ± 1.5 4.8	5.6 ± 0.4 1.3	8.4 ± 0.4 1.2	10.5 ± 0.8 2.5	25.6 ± 1.0 3.4
Stationary cases	11	8.0 ± 0.1 0.4	57.3 ± 1.3 4.3	5.9 ± 0.4 1.3	7.5 ± 0.4 1.4	11.4 ± 0.7 2.3	17.5 ± 0.6 2.1

Table VI Significance of difference (t-test)

Groups	Total protein	Albumin	Globulins			
			α_1	α_2	β	γ
BHL progressive/Normals	×××	×××	—	×××	××	—
BHL stationary/Normals	×	—	—	××	t	—
BHL progressive/BHL stationary	—	×××	—	×	×	—
Diss. parenchym. progressive/Normals	×××	×××	—	××	—	××
Diss. parenchym. stationary/Normals	—	××	—	××	××	—
Diss. parenchym. progressive/ Diss. parenchym. stationary		—	—	×	—	×
Fibrosis progressive/Normals	×××		—	×××	—	×××
Fibrosis stationary/Normals	××	×	—		××	×
Fibrosis progressive/Fibrosis stationary	×		—	—	—	×××

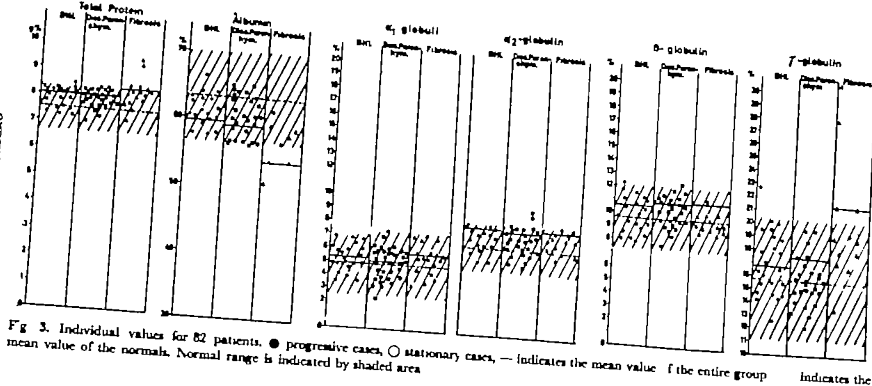


Fig 3. Individual values for 82 patients. ● progressive cases, ○ stationary cases, — indicates the mean value f the entire group indicates the mean value of the normals. Normal range is indicated by shaded area

Table V. Values for total protein and paper electrophoresis of serum. Mean value ± SE of mean and (below) SD are given

Clinical group	Total no. of cases	Total protein (g/100 ml)	Albumin (%)	Globulins (%)			
				α_1	α_2	β	γ
Controls	25	7.5 ± 0.1 0.4	63.4 ± 0.7 3.3	4.8 ± 0.2 1.2	8.5 ± 0.2 0.9	9.6 ± 0.2 1.2	13.7 ± 0.5 2.3
BHL							
Whole group	26	8.0 ± 0.1 0.4	59.6 ± 0.7 3.7	5.2 ± 0.2 1.2	8.0 ± 0.3 1.6	10.5 ± 0.4 1.9	16.7 ± 0.5 2.4
Progressive cases	10	8.2 ± 0.1 0.3	56.7 ± 1.3 3.8	5.6 ± 0.3 1.1	8.8 ± 0.5 1.6	11.6 ± 0.7 2.3	17.4 ± 0.8 2.5
Stationary cases.	16	7.9 ± 0.1 0.4	61.4 ± 0.5 2.1	4.9 ± 0.3 1.2	7.3 ± 0.3 1.4	9.8 ± 0.3 1.5	16.4 ± 0.6 2.3
Dez. parenchyma							
Whole group	34	8.0 ± 0.1 0.5	59.1 ± 0.7 4.0	5.0 ± 0.2 1.2	7.8 ± 0.3 1.5	10.8 ± 0.3 1.6	17.4 ± 0.6 3.5
Progressive cases	12	8.3 ± 0.2 0.6	57.6 ± 1.0 3.5	4.9 ± 0.4 1.4	8.5 ± 0.4 1.3	10.5 ± 0.5 1.7	19.2 ± 1.2 4.2
Stationary cases.	22	7.9 ± 0.1 0.4	60.3 ± 0.8 3.9	5.1 ± 0.2 1.1	7.4 ± 0.3 1.3	11.0 ± 0.3 1.6	16.3 ± 0.6 2.8
Fibrous							
Whole group	22	8.3 ± 0.1 0.6	53.8 ± 1.3 5.9	5.7 ± 0.3 1.3	8.0 ± 0.3 1.4	10.9 ± 0.5 2.4	21.5 ± 1.0 5.0
Progressive cases	11	8.7 ± 0.2 0.6	50.0 ± 1.5 4.8	5.6 ± 0.4 1.3	8.4 ± 0.4 1.2	10.5 ± 0.8 2.5	25.6 ± 1.0 3.4
Stationary cases	11	8.0 ± 0.1 0.4	57.5 ± 1.3 4.3	5.9 ± 0.4 1.3	7.5 ± 0.4 1.4	11.4 ± 0.7 2.3	17.5 ± 0.6 2.1

Table VI. Significance of difference (t-test)

Groups	Total protein	Albumin	Globulins			
			α_1	α_2	β	γ
BHL progressive/Normals	×××	×××	—	×××	××	—
BHL stationary/Normals.	×	—	—	××	—	—
BHL progressive/BHL stationary	—	×××	—	×	×	—
Dem. parenchym. progressive/Normals	× ×	×××	—	×××	—	××
Dem. parenchym. stationary/Normals	—	××	—	×	××	—
Dem. parenchym. progressive/ Dem. parenchym. stationary	×	—	—	×	—	×
Fibrous progressive/Normals	× ×	×××	—	×××	—	×××
Fibrous stationary/Normals	××	××	—	×	××	×
Fibrous progressive/Fibrous stationary	×	×××	—	—	—	×××

Table VII. Values for total protein in 10 controls and 10 patients with sarcoidosis at different posture. Mean value and SD are given

	Controls			Sarcoidosis		
	In bed after a night's rest	After 6 hrs in supine position	Diff.	In bed after a night's rest	After 6 hrs in supine position	Diff.
Total protein (g/100 ml)	7.2 ± 0.4	7.8 ± 0.3	+ 0.6	7.5 ± 0.3	8.6 ± 0.5	+ 1.1
Albumin (%)	60.9 ± 2.4	61.0 ± 2.2	+ 0.1	59.6 ± 2.5	58.2 ± 2.6	− 1.4
Globulins (%)						
α_1	5.2 ± 0.5	5.0 ± 0.6	− 0.2	3.4 ± 0.5	4.0 ± 0.4	+ 0.6
α_2	6.9 ± 0.5	6.6 ± 0.9	− 0.3	6.8 ± 0.8	8.0 ± 0.9	+ 1.2
β	10.3 ± 0.7	10.5 ± 0.8	+ 0.2	11.5 ± 1.6	11.4 ± 1.4	− 0.1
γ	16.7 ± 1.4	16.9 ± 1.2	+ 0.2	18.7 ± 1.5	18.4 ± 1.8	− 0.3

pulmonary tissue in a few BHL-cases with radiographically normal lungs (9, 14). Nevertheless, the BHL-group is probably the most uniform.

It is sometimes difficult to decide whether a case belongs to the group of patients with disseminated parenchymal lesions or to the fibrotic group. The healing of the sarcoid granuloma takes place through hyalinization and fibrosis. Even in apparently early cases histological examination shows a weft of fibrosis in the sarcoid tissue (13). In the disseminated parenchymal cases the lesions are miliary and usually show a tendency to cluster in the region of small lymphatic channels. In the fibrotic cases the infiltrates are more nodular, lesions in the interstitial tissue destroy the bronchioli, the small vessels and the alveoli. The trend to contract is pronounced. Practically all the fibrotic patients have also more or less marked dyspnoea. It would perhaps be possible to divide the patients with parenchymal lesions more accurately into groups through biopsies from the pulmonary tissue. Against this it may be objected that biopsy is not representative of the entire lung. The same biopsy often shows a varying histological picture. Furthermore lung biopsy can hardly be performed according to routine on persons who are usually clinically healthy. Svanborg (22) has shown moreover that when applying the same principles of classification there are characteristic differences between the various groups in respect to heart and pulmonary functions.

When judging whether a case is progressive or not both roentgenological and clinical findings have been taken into consideration, such as progressive enlargement of the lymph nodes, more pronounced parenchymal lesions, scar swellings, joint or eye symptoms. As previously mentioned this classification was done by the head and the staff of the lung department without their knowledge of the results of the protein analyses.

Through the history the duration of the disease can sometimes be estimated but this can be done only in the relatively few cases where the symptoms are so marked that the patient consults a physician. This applies especially to those cases whose onset takes place in the form of erythema nodosum, joint or eye symptoms. More commonly the lesions are dis-

covered at a routine roentgenogram of the lungs. In order to obtain, if possible, an objective standard for measuring the duration of the disease, when presenting the groups, two time factors have been included, "time after discovery" and "duration". "Time after discovery" signifies the time that has elapsed after the first positive roentgenogram. This information only has been furnished by some investigators (20) when estimating the period of the disease. The period of the disease estimated in this way is obviously too short. By "duration" is meant the time that has elapsed since the last normal roentgenogram of the lungs. Consequently this represents the longest possible period of the disease. On account of the nation-wide mass X ray surveys that are regularly performed in Sweden, such normal "predisease" radiograms are lacking only in 12 of the patients.

The normal values obtained for total serum protein and for paper electrophoresis agree well with those previously given in the relevant literature (2).

In earlier investigations on protein pattern in sarcoidosis, increased serum protein values have been reported throughout (3, 6, 7, 15, 16, 17). In this investigation the values for serum proteins were found to be elevated in patients with progressive lesions, but were either normal or only slightly increased in patients with stationary lesions. As a rule, it has been assumed that the observed increase in serum proteins is dependent upon an actual increase in the serum globulins. This also takes place in many cases. However, some of the patients with increased total protein values have proportionally quite a normal electrophoretic protein distribution. Consequently calculated in gram per cent, the albumin value should also be increased. Hence there are reasons for supposing that the serum protein increase in a number of patients is due to factors other than an actual increase in globulins.

Among other investigators Lange (8) has shown that the serum protein values are approximately 9 % higher for persons who are standing or walking than for those who are lying down. The mean increase in this study was 8.3 % in the controls and 14.5 % in the sarcoid cases. This difference is statistically significant. This means, that, at least in those cases with a normal electrophoretic protein distribution, one of the causes of the elevated total protein value observed may be due to this marked variation dependent on the posture. Among other factors, this, in its turn, may perhaps be connected with the pronounced orthostatic reactions frequently observed in patients with sarcoidosis (22).

Since, as has been shown, the relative protein distribution is independent of the individual's body position (table VI), the electrophoretic values obtained are given as relative percentages. The patients who took part in this investigation, like the controls, were all up and usually the test samples were withdrawn when they visited the outpatient department.

Most authors state only that there is an increase in γ-globulins in sarcoidosis. As is evident from the present tables an increase in α_2- and β-globulins is found in many cases without a concomitant increase in the γ-globulins.

The α_2-globulin increase which was most marked in the progressive cases will be discussed in detail in a subsequent article (1).

An increase in β-globulin occurred in several cases in all three groups. In the BHL-group, 5 of the 10 patients with progressive lesions had elevated β-glob-

ulins, whereas only one case with stationary lesions had an increased value. In the disseminated parenchymal group 2 of the 12 progressive cases and 7 of the 24 patients with stationary lesions had increased values. Of the fibrotic cases 3 of the 11 progressive cases and 5 of the 11 patients with stationary lesions had elevated values.

An increased amount of β-globulins is most frequently connected with an increased lipoprotein content as in the nephrotic syndrome bile duct obstruction etc.

In the BHL-group in no case was the elevated β-globulin value accompanied by hyperlipaemia or by signs of disturbed kidney function In the disseminated parenchymal group case No. 54 and in the fibrosis group all the patients with elevated β-globulins had cholesterol values exceeding 300 mg % Signs of disturbed kidney function (reduced concentration capacity with or without slightly increased non protein nitrogen content and generally only with trace of proteinuria) were observed in case No 54 and in 6 out of the 8 patients with hyper β-globulinaemia in the fibrotic group Five of the last-mentioned patients revealed also, periodically hypercalcaemia. In no case were there signs of bile duct obstruction serum bilirubin and the alkaline phosphatases were normal in all patients.

Consequently it is possibly a question of an increase of two different kinds of β globulins, one occuring in early cases and not accompanied by hyperlipaemia or signs of disturbed kidney function and the other accompanied by hypercholesterolaemia and usually in connection with disturbed kidney function

The increase in β-globulins without hyperlipaemia has aroused special interest, and will be dealt with in a subsequent work on sarcoidosis and concurrent erythema nodosum.

In the present investigation an increase in the mean value of γ-globulins could be definitely shown to exist only in the groups of patients with pulmonary parenchymal lesions (Group II and III) In the BHL-group there was only one patient who had increased γ-globulins (> mean value + 2 SD) In the disseminated parenchymal group 8 patients (24 %) had increased γ-globulins, and in the fibrosis group there were 12 (55 %) These results showing for the entire material a total of 21 cases (25 %) with hyper γ-globulinaemia, deviate from those usually reported in the literature. This may be partly explained by the fact that previously mainly patients with pulmonary parenchymal lesions were investigated Since, as a rule clinical data are not available it is not possible to compare the materials. Mather et al. (12) have divided their material consisting of 93 patients into groups of cases with only BHL, with BHL and concurrent pulmonary lesions, and with pulmonary infiltrations without hilar lymphomas. They found only ten patients in the whole material with hyper γ-globulinaemia, but do not state to which group these patients belong Fröhlich (4) has made a very detailed classification of his material according to the same principles as those applied in this material but he reported in the different groups an unspecified number of electrophoretic results for each patient and hence, it is not possible to obtain any conception of the individual values

Besides occuring in subacute-chronic infections an increase in γ-globulins is mainly found in connection with fibrous-productive processes such as hepatic cirrhosis and some collagenoses. The

frequent involvement of the liver in sarcoidosis has been referred to in the literature (12, 18) Mather et al (12) found in 93 patients 59 with sarcoid granulomas in the liver biopsy The frequency of positive liver biopsies was approximately 75 % in the groups with BHL with or without pulmonary paren chymal lesions, and about 59 % in the group with only parenchymal lesions. In 98 post mortem examinations in the literature hepatic lesions were found in 59 cases (60 %) Mather et al. did not find any correlation between hepatic damage and changes in the serum protein pattern.

The present investigation could not definitely answer the question to what extent concomitant liver damage had influenced the serum γ-globulin content. Serum bilirubin alkaline phosphatases, GOT and GPT were normal in all patients. In five patients with hyper γ-globulinaemia in the fibrous group liver function test with bromsulphthalein was without remark. Liver biopsy could only be carried out in exceptional instances. For 2 patients in the BHL-group the biopsies were without remark and in 4 cases the biopsies were positive In one patient in the disseminated parenchymal group a liver biopsy carried out in con nection with a gall bladder operation, showed sarcoid granulomas. In 4 other patients from this group the biopsies were normal. Later 2 patients from the fibrous group died Both of them had greatly increased γ-globulins At autopsy in both these cases the liver was macroscopically normal In one case there were microscopically isolated apparently healed granulomas, in the other the liver was histologically normal. Thus in this material no pparent connection between liver damage as it ppears in laboratory tests and hyper γ-globulinaemia was observed

It is worthy of note that in the group of patients with fibrotic pulmonary lesions 10 out of 22 cases (45 %) had normal γ-globulins. Among these patients there are several (Nos. 62, 64 68 and 80) with very marked pulmonary lesions. In all of these patients, however the roentgenograms have remained unchanged for a number of years. Two of these patients (Nos. 62 and 64) revealed, at electrophoretic examination two and three years before the present occasion, γ-globulins in excess of a relative 25 %

An increase in serum γ-globulins indicates a progressive fibrotic process. On the other hand normal γ-globulins do not exclude the presence of advanced fibrotic lesions, which then can be thought of, however as being at a "stationary" stage.

Summary

The total serum protein value and the electrophoretic protein pattern were de termined in 82 cases of sarcoidosis, in all of which the diagnosis was supported by biopsy The clinical material was classified in different groups according to the clinical course of the intrathoracic sarcoidosis. The results obtained were correlated with the different stages of the disease.

The significance of the increase in total serum protein which was manifest in the groups with progressive lesions was discussed The relationship to posture of the total serum protein value was compared between patients with sarcoidosis and controls. The increase of the serum proteins after the subjects had been up and about was significantly greater in the sarcoidosis group than in the control group. In both groups the electrophoretic

protein pattern was independent of posture.

In all the groups the mean value of relative albumin concentration was lowered

The mean value of α_2-globulin was elevated in all the groups.

An increase in β-globulin occurred in several cases of all the groups. The possible occurrences of two kinds of increase in β-globulin was discussed one with normal serum cholesterol value and without signs of concurrent kidney damage, and the other accompanied by hypercholesterolaemia and in the majority of cases, also with reduced kidney function

The mean value for γ-globulins was increased only in the groups with progressive lesions. In the other groups the mean value was normal Other points discussed were the correlation between the progressive pulmonary fibrosis and hyper γ-globulinaemia and the significance of the liver in connection with an increase in γ-globulin

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Studies in Sarcoidosis

II. Serum Proteinbound Carbohydrates

By

L. E. Böttiger and Renée Nordberg

During the last decade a number of reports have been published on serum proteinbound carbohydrates in different diseases (30). With regard to sarcoidosis, however, few facts are available (8, 15). Only a small number of sera from patients with sarcoidosis have been examined and the results are difficult to evaluate as information on clinical data are lacking.

In several investigations, mainly into rheumatoid arthritis, the good correlation between clinical activity and elevated glucoproteins in serum has been pointed out (9, 19, 23). To see whether the determination of serum proteinbound carbohydrates would help in evaluating the activity of the disease also in sarcoidosis we have studied serum glucoproteins in a large number of such patients. This study was prompted also by the finding of periodic-acid-Schiff (PAS) positive material in the sarcoid tissue (14, 26, 27). An attempt has been made to correlate the results with the different stages of the disease, and with other laboratory findings.

Submitted for publication September 12, 1963.

Material

Eighty-two cases of sarcoidosis with intrathoracic lesions were investigated. In all cases the diagnosis was supported by biopsy from lymph node. In a previous paper (11) an account was given of the classification of these patients into three groups according to the chest roentgenograms: 1) Patients with bilateral hilar lymphadenopathy without visible lung lesions (BHL). 2) Patients with disseminated parenchymal pulmonary lesions with or without concomitant hilar lymphadenopathy (diss. parenchym.). 3) Patients with fibrotic pulmonary lesions (fibrosis). In each of these groups the patients who showed signs of current progress (indicated by fat types in the tables) either roentgenologically or clinically were placed in a subgroup, according to criteria discussed in the previous paper (11).

Control sera were obtained from healthy hospital personnel (11).

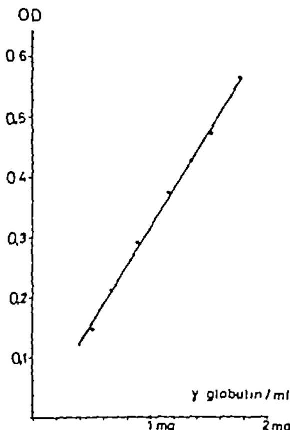


Fig 1 Extinction curve for hexoses determinations with Mokrasch method on different dilutions of γ-globulin

Methods

Proteinbound carbohydrates were determined after ethanol precipitation as follows

Hexoses with the anthrone reagent according to Mokrasch (10) (Hydrolysis for 25 min at 80°)

The mean value for proteinbound hexoses (107 mg/100 ml) is somewhat lower than the normal mean values previously given (111—123 mg/100 ml) (1 4 21 30)

Correction has been made for the colour which occurs as a result of the reaction between sulphuric acid and protein but no correction has been made for the presumed effect of the reaction tryptophan-carbohydrate anthrone. Tuller and Keding (28) using Graff's anthrone method (7) estimated normal values for serum proteinbound hexoses to be 15—20 % too low owing to tryptophan lowering the extinction.

The reaction tryptophan-carbohydrate-anthrone has its absorption maximum at 550 mμ, but interferes with Graff's method in the absorption at 630 mμ which is the maximum for the carbohydrate anthrone reaction. Using the Mokrasch-method also employed by us, which involves a weaker acid and a lower heating temperature than Graff's method, Sonnet (21) found the effect of tryptophan to be negligible

When performing hexose determinations with Mokrasch's method on different dilutions of a γ-globulin fraction containing 3 g tryptophan/100 g protein the extinctions were found to follow a straight line (fig 1). Consequently the absorption value of the hexoses seems to be independent of the tryptophan content

Hexosamines, according to Blix's modification of Elson-Morgan's method described by Svennerholm (24) and slightly modified by Böttiger and Carlson (4)

Sialic acids with resorcinol-Cu reagent according to Svennerholm (25) Th factor 0 95 was used to correct for colour from substances other than sialic acids.

Seromucoid, was determined as protein after precipitation according to Winzler (31)

Paper electrophoresis was performed as described in the previous paper (11)

E.S R. according to Westergren's method.

C-reactive protein was determined with the Schiefflin antigen

Experimental errors were calculated for normal and pathological sera The results are shown in table IV

Statistical calculations were made according to conventional methods (20) Significance of difference between groups and the magnitude of the correlation coefficient was tested by the t test. The degree of probability was designated as follows

P ⩽ 0.05 probably significant (x)
P ⩽ 0 01 significant (xx)
P ⩽ 0 001 highly significant (xxx)

Individual values outside the normal ranges (the mean value ± 2 SD) are indicated by Italics in the tables.

Results

The individual values for the amount of serum proteinbound hexoses, hexosamines, and sialic acids and the sum of

Fig. 2. Individual values for 82 patients. ● progressive cases, ○ stationary cases. Normal range is indicated by shaded area.

these values, the seromucoid value, the serum α_2-globulin content, E.S.R. and the result of the CRP test are given in tables I—III and in figs. 2 and 3. The mean values in the different sarcoidosis groups and in the control material are given in table V.

The following results should be particularly emphasized:

The individual values for *protein-bound hexoses, hexosamines, sialic acids* and *seromucoid* were, with a few exceptions, elevated in the cases with clinical and roentgenological signs of *progress*. In the *stationary* cases the values were either normal or slightly elevated.

In all the sarcoidosis groups, except the one with stationary BHL-cases, the mean values for the different glucoproteins were significantly higher than those for the normal cases. The mean value of

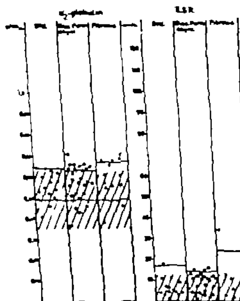


Fig. 3. Individual values for 82 patients. Symbols as in fig. 2.

Table I Group I Patients with bilateral hilar lymphadenopathy

Case	α globulin (g/100 ml)	Total glucoproteins (mg/100 ml)	Seromucoid (mg/100 ml)	ESR	CRP
1	*0.64*	*311*	*118*	*64*	+
2	*0.76*	*359*	*187*	*54*	+
3	0.60	257	*108*	4	–
4	*0.64*	*318*	*101*	*23*	+
5	*0.88*	*310*	*105*	*18*	+
6	*0.66*	257	*93*	5	–
7	*0.92*	*357*	*129*	*47*	+
8	0.46	*272*	85	*30*	–
9	*0.82*	*304*	*119*	*15*	–
10	0.60	269	*96*	*7*	–
11	*0.67*	*305*	*110*	*13*	–
12	0.62	*303*	*119*	*98*	+
13	*0.90*	*89*	*101*	*19*	+
14	*0.70*	246	78	3	–
15	0.52	281	*98*	4	–
16	*0.63*	248	89	7	–
17	0.57	265	67	6	–
18	*0.79*	268	79	9	–
19	0.49	264	*100*	11	–
20	0.41	235	84	4	–
21	0.54	236	78	1	–
22	0.58	226	77	3	–
23	*0.74*	278	80	*22*	
24	0.50	281	*93*	*20*	–
25	0.53	250	83	4	–
26	0.55	264	*100*	11	–

Table II Group II Patients with disseminated parenchymal pulmonary lesions

Case	α_2 globulin (g/100 ml)	Total glucoproteins (mg/100 ml)	Seromucoid (mg/100 ml)	ESR	CRP
27	0.56	232	90	4	–
28	*0.71*	275	*97*	7	+
29	*0.83*	*301*	83	*39*	–
30	*0.77*	287	87	*34*	–
31	*0.64*	285	*91*	*21*	–
32	*0.70*	277	*120*	*35*	–
33	0.63	262	*94*	12	–
34	*0.65*	241	*92*	12	–
35	0.56	253	73	11	–
36	0.45	*293*	90	*15*	–
37	*0.66*	*290*	*100*	5	–
38	*0.94*	*328*	*108*	*19*	–
39	0.59	239	83	2	–
40	*0.88*	*356*	*162*	10	(+)
41	*0.64*	*321*	*125*	12	(+)
42	0.57	248	*105*	11	–
43	0.51	237	*95*	11	–
44	*0.65*	266	*92*	4	–
45	0.57	248	70	3	+
46	0.52	287	*112*	12	(+)
47	*0.66*	*302*	*100*	*18*	(+)
48	*0.76*	*309*	*125*	*35*	+
49	*0.70*	*326*	*125*	*13*	+
50	*0.64*	281	*95*	*29*	–
51	0.58	260	75	*4*	–
52	*0.79*	*305*	*112*	8	–
53	0.50	265	78	3	(+)
54	*0.46*	*333*	*120*	*16*	–
55	0.43	251	*93*	3	–
56	0.59	260	*91*	1	–
57	0.52	*289*	*98*	*13*	(+)
58	0.62	*294*	*108*	*28*	–
59	0.57	*325*	*138*	*15*	–
60	0.46	*292*	*127*	7	–

seromucoid was significantly elevated in *all the sarcoidous groups* (table VI).

The mean values of the glucoproteins in the progressive groups were significantly higher than those in the groups with stationary lesions. This applies also to the mean seromucoid values with the exception of the fibrous groups (table VI).

Generally the *α_2 globulin* values were elevated in the progressive cases. The differences in the mean values between the progressive and the stationary groups were not significant, however, except in the cases of disseminated pulmonary lesions.

E.S.R. was elevated in the patients with progressive lesions and, as a rule, normal in the other cases.

The *CRP-test* was generally positive in the patients with progressive lesions.

The E.S.R.-value, like the α_1-globulins showed good correlation with the total amount of serum glucoproteins (in both cases $P < 0.001$)

The ratios hexoses/hexosamines/sialic acids were the same in the different groups and in the control material.

Discussion

Hitherto the patho-physiological significance of the serum proteinbound carbohydrates is unknown. In a large number of papers (30) it has been shown that increase in proteinbound carbohydrates occurs, inter alia, in febrile states of different origin, in cases of tissue destruction, malignant tumours, etc. A decrease in the seromucoid content has been demonstrated in severe cases of liver parenchymal damage. The investigations especially of Werner (29) Boström (2) and Spiro (22) have shown that the formation of proteinbound carbohydrates takes place mainly in the liver

The connection between elevated serum proteinbound carbohydrates and the colouring of the tissue on applying PAS has been discussed by Teilum (27) and Shetlar (16) among others. Teilum believes that the serum proteinbound carbohydrates are derived, at least partially from the tissue mucopolysaccharides, whereas Shetlar regards the connection as probable, but does not find any definite support for the formation of serum proteinbound carbohydrates in the tissue. He considers as the most likely hypothesis, that the increase of proteinbound carbohydrates in serum like the increased polysaccharide content in the tissue, represents a systemic response to damage or to tissue proliferation. Böttiger (3) found in a collection of cases of renal

Table III. Patients with fibrotic pulmonary lesions

Case	α_1 globulin (g/100 ml)	Total gluco-proteins (mg/100 ml)	Sero-mucoid (mg/100 ml)	ESR	CRP
61	0.75	321	120	34	+
62	0.68	288	97	32	−
63	0.55	281	96	13	+
64	0.58	226	74	2	−
65	0.66	263	90	6	−
66	0.79	307	112	17	+
67	0.71	357	120	35	+
68	0.75	294	110	12	+
69	0.61	271	86	6	−
70	0.57	339	129	35	+
71	0.63	328	138	52	+
72	0.61	255	81	8	−
73	0.71	346	138	28	+
74	0.83	—		51	Not made
75	0.83	428	180	110	+
76	0.75	285	108	18	+
77	0.46	293	106	3	−
78	0.86	337	132	34	+
79	0.62	305	120	3	−
80	0.56	277	100	12	−
81	0.68	307	120	18	+
82	0.56	254	70	15	−

Table II. Experimental errors (mg/100 ml)

	Hexoses	Hexosamines	Sialic acids	Seromucoid
Normal sera (n = 25)				
Mean value	106	88	56	71
S	2.4	2.2	1.0	3.6
S (%)	2.3	2.5	1.9	5.0
Pathological sera (n = 25)				
Mean value	174	155	99	263
S	2.5	2.4	1.9	9.0
S ( )	1.4	1.5	1.9	3.4

$$S = \sqrt{\frac{\Sigma(d^2)}{2n}}$$

Table I Group I Patients with bilateral hilar lymphadenopathy

Case	α_2 globulin (g/100 ml)	Total gluco-proteins (mg/100 ml)	Sero-mucoid (mg/100 ml)	ESR	CRP
1	*0.64*	*311*	*118*	*64*	+
2	*0.76*	*359*	*187*	*54*	+
3	0.60	257	*108*	*4*	–
4	*0.64*	*318*	*101*	*23*	+
5	*0.88*	*310*	*105*	*18*	+
6	*0.66*	257	*93*	5	–
7	*0.92*	*357*	*129*	*47*	+
8	0.46	272	85	*30*	–
9	*0.82*	*304*	*119*	*15*	–
10	0.60	269	*96*	*27*	–
11	*0.67*	*305*	*110*	*13*	–
12	0.62	*303*	*119*	*28*	+
13	*0.90*	*289*	*101*	*19*	+
14	*0.70*	246	78	3	–
15	0.52	281	*98*	4	–
16	*0.63*	248	89	7	–
17	0.57	265	67	6	–
18	*0.69*	268	79	9	–
19	0.49	264	*100*	11	–
20	0.41	235	84	4	–
21	*0.54*	236	78	*1*	–
22	0.58	226	77	3	–
23	*0.74*	278	80	*22*	–
24	0.50	281	*93*	*20*	–
25	0.53	250	83	4	–
26	0.55	264	*100*	11	–

Table II Group II Patients with disseminated parenchymal pulmonary lesions

Case	α_2 globulin (g/100 ml)	Total gluco-proteins (mg/100 ml)	Sero-mucoid (mg/100 ml)	ESR	CRP
27	0.56	232	90	4	–
28	*0.71*	275	*97*	7	+
29	*0.83*	*301*	83	*39*	–
30	*0.77*	287	87	*34*	–
31	*0.64*	283	*91*	*21*	–
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33	0.63	262	*94*	12	–
34	*0.65*	241	*92*	12	–
35	0.56	253	73	11	–
36	0.45	*293*	90	*15*	–
37	*0.66*	*290*	*100*	5	–
38	*0.94*	*328*	*108*	*19*	–
39	0.59	239	83	*2*	–
40	*0.88*	*356*	*162*	10	(+)
41	*0.64*	*321*	*125*	12	(+)
42	0.37	248	*105*	11	–
43	0.51	257	*95*	11	–
44	*0.65*	266	*92*	4	–
45	0.57	248	70	3	+
46	0.52	287	*112*	12	(+)
47	*0.66*	*302*	*100*	*18*	(+)
48	*0.76*	*309*	*125*	*35*	+
49	[illegible]	[illegible]	*125*	*13*	+
50	*0.64*	281	*95*	*29*	–
51	0.58	260	75	*24*	–
52	*0.79*	*305*	*112*	8	–
53	0.50	265	78	3	(+)
54	*0.66*	*333*	*120*	*16*	–
55	0.43	251	*93*	3	–
56	0.59	260	*91*	1	–
57	0.52	*289*	*98*	*13*	(+)
58	0.62	*294*	*108*	*28*	–
59	0.57	*325*	*138*	*15*	–
60	0.46	*292*	*127*	7	–

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The mean values of the glucoproteins in the progressive groups were significantly higher than those in the groups with stationary lesions. This applies also to the mean seromucoid values, with the exception of the fibrosis groups (table VI)

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Discussion

Hitherto the patho-physiological significance of the serum proteinbound carbohydrates is unknown. In a large number of papers (30) it has been shown that increase in proteinbound carbohydrates occurs, inter alia, in febrile states of different origin, in cases of tissue destruction, malignant tumours, etc. A decrease in the seromucoid content has been demonstrated in severe cases of liver parenchymal damage. The investigations especially of Werner (29) Boström (2) and Spiro (22) have shown that the formation of proteinbound carbohydrates takes place mainly in the liver

The connection between elevated serum proteinbound carbohydrates and the colouring of the tissue on applying PAS has been discussed by Teilum (27) and Shetlar (16) among others. Teilum believes that the serum proteinbound carbohydrates are derived, at least partially from the tissue mucopolysaccharides, whereas Shetlar regards the connection as probable but does not find any definite support for the formation of serum proteinbound carbohydrates in the tissue. H considers as the most likely hypothesis that the increase of proteinbound carbohydrates in serum, like the increased polysaccharide content in the tissue represents a systemic response to damage or to tissue proliferation. Böttiger 3) found in a collection of cases of renal

Table III Patients with fibrotic pulmonary lesions

Case	α_1 globulin (g/100 ml)	Total glucoproteins (mg/100 ml)	Seromucoid (mg/100 ml)	ESR	CRP
61	*0.75*	*321*	*120*	*34*	+
62	*0.68*	288	*97*	*52*	−
63	0.55	281	*96*	*13*	+
64	0.38	226	74	2	−
65	*0.66*	263	90	6	−
66	*0.79*	307	*112*	*17*	+
67	*0.71*	357	*120*	35	+
68	*0.75*	*294*	*110*	12	+
69	0.61	271	86	6	−
70	0.57	339	*129*	55	+
71	*0.69*	*328*	*128*	*52*	+
72	0.61	255	81	8	−
73	*0.71*	*346*	*138*	*38*	+
74	*0.83*	—		*51*	Not made
75	*0.83*	*428*	*100*	*110*	+
76	*0.75*	293	*108*	*18*	+
77	0.46	293	*106*	3	−
78	*0.86*	337	*152*	*34*	+
79	0.62	305	*120*	3	−
80	0.56	277	*100*	12	−
81	*0.68*	307	*120*	*18*	+
82	0.56	254	70	*15*	+

Table IV Experimental errors (mg/100 ml)

	Hexoses	Hexosamines	Sialic acids	Seromucoid
Normal sera (n = 25)				
Mean value	106	68	56	71
S	2.4	2.2	1.0	3.6
S (%)	2.3	2.5	1.9	5.0
Pathological sera (n = 25)				
Mean value	174	155	90	263
S	2.5	2.4	1.8	9.0
S (%)	1.4	1.5	1.9	3.4

$$S = \sqrt{\frac{\Sigma(d^2)}{2n}}$$

Table 1 Proteinbound carbohydrates Mean value ± SE of mean and (below) SD are given

Clinical group	No. of cases	Gluco-proteins (mg/100 ml) Hexoses (H)	Hexosamines (HA)	Sialic acids (SIA)	Total	Seromucoid (mg/100 ml)	α_2-globulin (g/100 ml)	ESR	Ratios H/HA/SIA
Controls	25	106.6	86.0	54.5	247.1	71.4	0.49	<12	1.9/1.6/1.0
		±1.5	±1.7	±0.8	±3.7	±1.9	±0.01		
		7.6	8.4	4.1	18.5	9.4	0.07		
BHL									
Whole group	26	119.8	96.4	62.9	279.1	93.0	0.64	17	1.9/1.5/1.0
		±2.6	±2.6	±1.8	±6.7	±4.7	±0.03		
		13.4	13.2	9.3	34.2	23.8	0.13		
Progressive cases	10	132.8	107.1	70.5	310.4	115.3	0.72	30	1.9/1.5/1.0
		±2.9	±4.1	±2.6	±9.0	±8.9	±0.04		
		9.2	13.1	8.3	28.6	28.1	0.13		
Stationary cases	16	111.7	89.6	58.1	259.4	88.9	0.59	10	1.9/1.5/1.0
		±2.0	±1.9	±1.6	±4.9	±3.4	±0.03		
		7.9	7.7	6.[illegible]	19.5	13.5	0.11		
Dis parenchyma									
Whole group	34	120.3	100.	62.5	283.5	101.6	0.63	14	1.9/1.6/1.0
		±2.4	±1.9	±1.3	±5.2	±3.4	±0.02		
		13.8	11.1	7.4	30.5	20.0	0.12		
Progressive cases	12	132.8	109.8	69.0	311.6	116.2	0.71	19	1.9/1.6/1.0
		±3.4	±2.2	±2.2	±6.9	±6.2	±0.03		
		11.6	7.7	7.5	23.8	21.5	0.12		
Stationary cases	22	113.4	95.8	58.9	268.1	93.6	0.58	12	1.9/1.6/1.0
		±2.0	±2.0	±0.9	±4.6	±3.0	±0.02		
		9.5	9.4	4.3	21.5	14.[illegible]	0.10		
Fibrosis									
Whole group	21	127.2	107.4	68.7	303.3	110.3	0.67	24	1.9/1.6/1.0
		±4.0	±3.5	±2.3	±9.4	±7.7	±0.03		
		18.5	16.2	10.5	44.0	35.0	0.12		
Progressive cases	10	138.4	117.2	73.0	328.6	123.5	0.73	37	1.9/1.6/1.0
		±5.3	±5.2	±3.6	±13.6	±7.6	±0.03		
		16.6	16.3	11.4	42.9	23.9	0.10		
Stationary cases	11	117.1	98.5	64.8	280.4	108.2	0.60	12	1.8/1.5/1.0
		±4.2	±3.0	±2.5	±9.5	±6.0	±0.03		
		14.0	9.9	8.3	31.7	20.0	0.11		

carcinoma a probable correlation between the occurrence of PAS-positive reaction in the tumour tissue and elevated proteinbound carbohydrates in serum. No indications were, however found of glucoprotein synthesis in the tumour cells.

Also in sarcoidosis the PAS-positive reaction in the tissue and the serum pro-

Table VI Significance of difference (t-test)

Groups	Hexoses	Hexosamines	Sialic acids	Seromucoid	α_2-globulin
BHL-progressive/Controls	×××	×××	×××	×××	×××
BHL-stationary/Controls	×	—	—	×××	××
BHL-progressive/BHL-stationary	×××	×××	×××	××	×
Diss. parenchym. prog./Controls	×××	×××	×××	×××	×××
Diss. parenchym. stat./Controls	××	×××	×××	×××	××
Diss. parenchym. prog./Diss. parenchym. stat.	×××	×××	×××	×××	××
Fibrosis progressive/Controls	×××	×××	×××	×××	×××
Fibrosis stationary/Controls	××	×××	×××	×××	××
Fibrosis progressive/Fibrosis stationary	××	××	—	—	×

teinbound carbohydrates appear to be correlated, as the PAS-positive reaction of the pathological tissue is more marked in progressive than in stationary cases (13). The highest individual values of serum proteinbound carbohydrates are found in the apparently very active cases. (Cf. i. a. cases Nos. 1, 2, 7, 67 and 75). On the other hand, the majority of the stationary cases have normal serum proteinbound carbohydrates. This correlation between the PAS-positive tissue reaction and the serum proteinbound carbohydrates does not necessarily imply that the glucoproteins are formed in the pathological tissue. No attempt has been made to elucidate this question.

I[illegible] the fibrosis group there are several cases with pronounced but stationary pulmonary lesions. Most of these patients ha[illegible] normal content of serum proteinbound carbohydrates. In earlier studies (16, 17, 18) the connection has been po[illegible]ted out between the increase in serum protei[illegible]bound carbohydrates [illegible]nd tissue proliferation and breakdown respectively. Consequently in cases with normal serum proteinbound carbohydrates the fibrotic process ma[illegible] be regarded a[illegible] terminated. These patients, who display a picture of stationary fibrosis, also have a normal content of γ-globulins in serum. In accordance with the discussion in the previous paper (11) the fibrosing process may also from that point of view be considered completed and the roentgenogram revealing fibrosis as representing a cicatricial cond[illegible]tion.

The mean values for the total amount of serum glucoproteins, (hexoses, hexosamines and sialic acids) in the three groups of patients with stationary lesions do not differ statistically from each other nor do the mean values between the groups with progressive lesions. The composition of the three main groups, with a higher percentage of progressive cases in the fibrosis group, account for the apparent increase in the mean values of the glucoproteins for the whole of this group in comparison with the other groups (fig. 2). This indicates necessity of taking into account the activity of the disease-process when evaluating the serum proteinbound carbohydrates at a certain given [illegible]tage.

The ratios hexoses/hexosamines/sialic acids are the same in the control material

and in the different sarcoidosis groups. Consequently the increase of the different carbohydrates is parallel.

In general the increase in seromucoid is parallel to the increase of proteinbound hexoses, hexosamines and sialic acids. In electrophoresis at pH 8.6 the main part of the seromucoid (orosomucoid) migrates with the a_1-globulins. With paper electrophoresis as used here no increase of a_1 globulins in any group was found to be statistically significant. On the other hand zone electrophoretic investigations in polyvinyl chloride have shown the a_1-globulin fraction to be increased (12). This observation regarding the difference between paper electrophoresis and zone electrophoresis in polyvinyl chloride has also been made earlier (3). It is probably due to the possibility in zone electrophoresis with both protein and carbohydrate analyses, to divide the fractions so that more of the area between the albumin and a_1-globulin fraction correctly is brought to the a_1-globulins.

In inflammatory processes the connection between clinical signs of the activity of the disease, the E.S.R. and the CRP test has been known for a long time. As cases with concurrent diseases were excluded from the material the elevated E.S.R. and positive CRP test may be regarded as an expression of the activity of the sarcoidosis. The correlations between E.S.R. positive CRP test and observed roentgenological signs of progress were good (table I—III).

The positive correlation between the E.S.R. value and the serum proteinbound carbohydrates has been mentioned by several authors (5, 6, 19). A positive correlation was found to exist in the present investigation between the E.S.R. value and the glucoproteins (estimated as the sum of proteinbound hexoses, hexosamines and sialic acids) and similarly between the E.S.R. and the seromucoid value, and between E.S.R. and the serum a_1-globulins.

The results have demonstrated that elevated serum proteinbound carbohydrates cannot be used in establishing the diagnosis of sarcoidosis. They are good indicators of disease activity. The small increase in total content, which occurs in stationary cases, may be a sign that there is a certain degree of activity although so slight that it cannot be demonstrated roentgenologically.

Summary

In 82 cases of sarcoidosis the total amount of serum proteinbound carbohydrates, (estimated as hexoses, hexosamines and sialic acids) the seromucoid content, the a_1-globulin value, the E.S.R. and the C-reactive protein reaction were determined.

The results were correlated with the different stages of the disease.

There was good correlation between clinical activity and the increase of proteinbound carbohydrates, as it was between clinical activity and the other parameters.

The results have demonstrated that elevated serum proteinbound carbohydrates cannot be used in establishing the diagnosis of sarcoidosis. They are, however, good indicators of disease activity.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Comparison of Hemosiderin Estimation in Bone Marrow Sections and Bone Marrow Smears

By

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Over a decade ago Rath and Finch (12) introduced into clinical work the technique of examination of bone marrow smears for hemosiderin. Subsequently many authors found this simple method useful for estimation of iron stores and for separating other anemias from iron-deficiency anemia (6 13 14). Modifications of the original technique have been adopted by several authors. Stevens et al. (15) recommended the examination of unstained thick smears in which hemosiderin appears as golden-yellow refractile granules. Most authors, however use stained permanent preparations. The staining of bone marrow smears with the Prussian-blue reaction for determination of hemosiderin and examination of sideroblasts has been in routine use at this laboratory and has given satisfactory results (6 13). However many investigators prefer to use bone marrow sections instead of smears (1 ... 8 16 19) and some of them consider the examination of smears unreliable (17 19).

The main objection to the use of bone marrow smears is that the distribution of hemosiderin in the bone marrow is not uniform. It is therefore essential to use specimens with a representative yield of reticular cell elements if the presence of hemosiderin is to be excluded with reasonable certainty. However as stated by these authors (17 19) it is hard or impossible to evaluate the volume and cellularity of the marrow sample in a smear preparation. With the section technique on the other hand the volume of the aspirated marrow is readily estimated being proportional to the area examined (2). The second advantage of the section technique is that the location of iron with respect to the tissue cell can more readily be ascertained. However the use of smears has other advantages. The method is simpler and not so time-consuming. Artefacts are less common if fresh reagents and clean glassware are used. The most important advantage of the smear technique is that it enables

Submitted for publication September 16, 1963.

find any difference between the modifications of Lison and Hutchison, and these two do not with certainty differ from the Turnbull blue method. On the other hand, they give a considerably better yield and more distinctive color than the Wicklein-Falkenberg modification. If, according to Hutchison, the temperature is raised to about 55 C during the staining, the reaction will occur more rapidly and will seem to be fully evident within five minutes. But if the temperature is raised to over 60° C, a blue green discoloration of the reagent appears and precipitation in the material increases.

Grading of histiocytic iron in fixed sections

The sections were examined with 600 × magnification.

Grade 0 no iron or only obviously extracellular precipitation artefacts. Trace isolated very fine granules in the whole preparation, often difficult to determine whether intracellular or not. Grade I definite intracellular iron in single cells (scarce material) or several cells (abundant material). Grade II moderate amount of histiocytic iron. On the average one or several cells in each field of vision if the cellularity is good. Grade III abundant amount of histiocytic iron in several cells in each field of vision with some lumps. Grade IV very abundant amounts of histiocytic iron which to great degree consists of coarse crusts and lumps.

Comments on the system of grading

The method involves several sources of error. The amount of material available is variable and the iron-carrying histiocytes are always unevenly distributed. Due to this and unless there is a large amount of material, the negative findings or the grading of positive findings will be unreliable. It is not possible to avoid precipitation artefacts completely with the Prussian-blue method. These may be partly caused by contaminations during the histochemical preparation, but also by the oxidation of the ferrous iron in the reagent to ferric iron.

The grading is rather schematic, but due to the facts mentioned above it is useless to require greater exactitude.

Material

A total of 300 bone marrow punctures from 265 patients was examined. Samples which were found quantitatively unsatisfactory by one of the investigators were eliminated regardless of the result.

The control subjects (31 cases) were patients with hemoglobin values exceeding 13.5 g % for men and 11.5 g % for women, normal blood-indices, serum iron, TIBC and ESR and without signs of actual infection.

The patients diagnosed as having iron-deficiency anemia (34 cases) had a low serum iron, high unsaturated iron-binding capacity (UIBC) and, when more pronounced a lowered mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). They all had record of chronic blood loss or had clinical signs of malabsorption. They reacted favorably to adequate iron therapy. Their iron stores could merely on clinical grounds be judged as depleted.

The group of acute hemorrhagic anemia (31 cases) consisted mainly of patients with recent gastrointestinal bleedings. These patients had various degrees of iron loss but their iron stores could not be clinically established because they were not observed while in a steady state.

The group of infectious disease without anemia (24 cases) comprised patients with rheumatoid arthritis, chronic liver disease, malignancy and patients with high ESR rate and inexplicable fever. Some of these patients had hypoferremia but only in two cases was the UIBC above 300 µg %.

The group of infectious-toxic and hemolytic anemia (68 cases) consisted of patients suffering from active rheumatoid arthritis, chronic kidney insufficiency, megaloblastic and hemolytic anemia, leukemia and anemia associated with malignancy without signs of external blood loss. The degree of anemia

the simultaneous evaluation of bone marrow cytology and sideroblasts. The latter may sometimes help in the evaluation of iron stores (6, 15).

As no comparison of the hemosiderin content in bone marrow sections and smears has been published, our studies of a comprehensive material with the simultaneous use of both methods are reported.

Methods

The blood-indices, serum iron and total iron binding capacity (TIBC) were examined in all cases. The serum iron was determined by the methods of Brochner-Mortensen (3) or Laurell (9) and the TIBC by a slight modification of Ramsay's method (11).

The marrow was aspirated in the usual manner from the sternum. A Wihman needle No. 18, suitable for a Luer Lock syringe, was used. The suction was performed with a 10 or 20 ml all-glass syringe. During suction the needle was gently moved round in the marrow cavity. By this manipulation satisfactory marrow volumes were obtained. The content was then ejected onto two or more slides from which the peripheral blood was removed with filter paper. In addition to conventional smears for morphological evaluation and sideroblast counting, thick smears and so-called squash preparations were made for hemosiderin determination. The rest of the bone marrow particles were fixed in 10 % neutral formaldehyde reagent grade.

Different investigators independently evaluated the bone marrow smears and the fixed sections without access to essential clinical data.

Staining and estimation of hemosiderin and sideroblasts in the bone marrow smears were done by the methods earlier described (6).

Grading of hemosiderin in smear preparations

The slides were examined with a magnification of 1,200 × under oil immersion.

Grade 0: no hemosiderin granules in the whole preparation. Trace: one or a few granules in the whole preparation. Grade 1: fine granules in about every third or fourth oil-immersion field. Grade 2: rather more heavy granules in about every second or third immersion field. Grade 3: hemosiderin granules in every immersion field in one or more cells. Grade 4: massive hemosiderin deposits with clumps and heavy granules.

In our earlier work we paid attention only to the intrareticular hemosiderin, as the extracellular iron was hard to distinguish from contaminations. With growing experience we learned to separate the fine hemosiderin granules from the amorphous artefacts. We therefore recorded and classified separately the intra- and extracellular hemosiderin in thin smears as well as in thick and squash preparations.

Histotechnical methods

The bone marrow was fixed in 10 % neutral formaldehyde, usually for 24 hours but in some cases for up to 72 hours. It was embedded in paraffin in the usual manner and made into 3 μ thick sections, which were subsequently stained with hematoxylin-van Gieson, eosin and Giemsa. The staining of hemosiderin was carried out by the Prussian-blue method according to the modification of Hutchison (8).

Discussion of the Prussian-blue method

This method has been used by various investigators in a number of modifications in which the concentrations of the reagents, the type of acid as well as the staining time and temperature vary. Lison (10) used equal parts of 2 % HCl and 2 % $K_4Fe(Cn)_6$ and stained at room temperature. Hutchison (8) used equal parts of 4 % HCl and 4 % $K_4Fe(Cn)_6$ and stained for 10 minutes at 56 °C. Bunting (4) tried different concentrations of reagents but found that the method used by Lison gave the best result.

We have compared, in addition to these two methods, the Turnbull blue method and an older one by Wicklein-Falkenberg (18) which uses considerably lower concentrations of $K_4Fe(Cn)_6$. At the same temperature, we cannot

find any difference between the modifications of Lison and Hutchison, and these two do not with certainty differ from the Turnbull blue method. On the other hand, they give a considerably better yield and more distinctive color than the Wicklein-Falkenberg modification. If, according to Hutchison, the temperature is raised to about 55° C during the staining, the reaction will occur more rapidly and will seem to be fully evident within five minutes. But if the temperature is raised to over 60° C, a blue green discoloration of the reagent appears and precipitation in the material increases.

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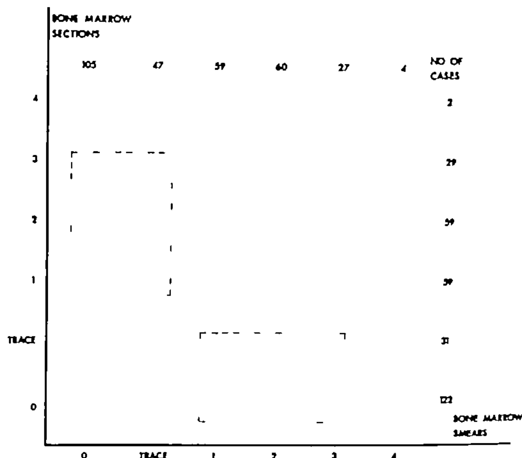


Fig 1 The relation of simultaneously determined stainable iron in bone marrow sections and smears. Only intracellular iron has been taken into account. Cases which show discrepancies between the methods are enclosed by a dotted line For details see text.

varied widely in this group. The TIBC was usually depressed

The group of infectious-toxic anemia with probably concomitant iron deficiency (23 cases) was an ill-defined group The patients had a moderate or rather severe anemia with a history of blood loss or malnutrition but did not respond to iron therapy

The group treated with parenteral iron (15 cases) comprised cases of chronic iron-deficiency anemia, examined shortly after the parenteral administration of 800—1 500 mg colloidal iron.

The last group (24 cases) comprised cases of polycytemia vera with hypochromic blood indices and a rather low serum iron concentration and cases of anorexia nervosa, chronic gastritis with a history of vomiting but without anemia.

Results and discussion

Fig 1 shows a direct comparison between the simultaneous determinations of hemosiderin in fixed sections and smear preparations. In this comparison only intrareticular iron was taken into account Thus if only extracellular iron had been found in a smear preparation it was regarded as devoid of hemosiderin. As the most important thing for the clinical evaluation and for the differential diagnosis is to know if iron is present or not, we deemed it a discrepancy if the findings were trace or 0 by one method and grade 1 or more by the other method. If on the other hand hemosiderin is present, the relative amount is of little clin

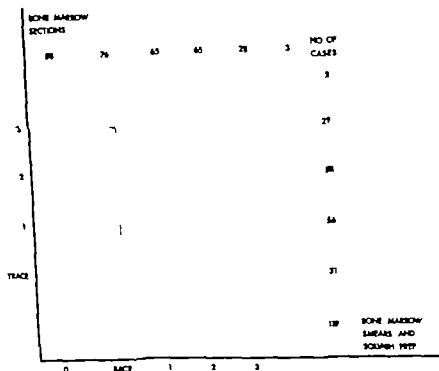


Fig 2 As in fig. 1 but here intra- and extra-cellular iron has been evaluated and in addition thick preparations examined. See text.

ical importance as iron deficiency can t any rate be excluded. Thus a minor divergency between the grades of hemosiderin present was not regarded as a discrepancy

As can be seen from the figure, in 24 cases (8) where hemosiderin-carrying histiocytes were found in fixed sections none or only trace was found in smear preparations On the other hand, in 25 cases (8) intrareticular ron was found the smears while none or only a trace ppeared in the sections.

In fig 2 the results are presented in the same manner as in fig. 1 but here attention has been paid to the extracellula hemosiderin in smea preparations in addition to the findings in the thick and squash preparations. Thus the result was regarded as positive if either reticular or extracellular hemosiderin was found in any of the smear or squash preparations.

In this figure only 14 cases (5 %) were positive in sections and negative in smear preparations, and 30 cases (10 %) were negative in sections but positive in smear preparations. In other words, when both free hemosiderin and thick preparations were evaluated, an additional 15 cases (5 %) became positive for hemosiderin. This is in accordance with the experience of Beutler et al (2)

If the classes 0 and trace are taken together then we find in fig 1 a quite similar number of cases, no matter which method of examination was used But one can

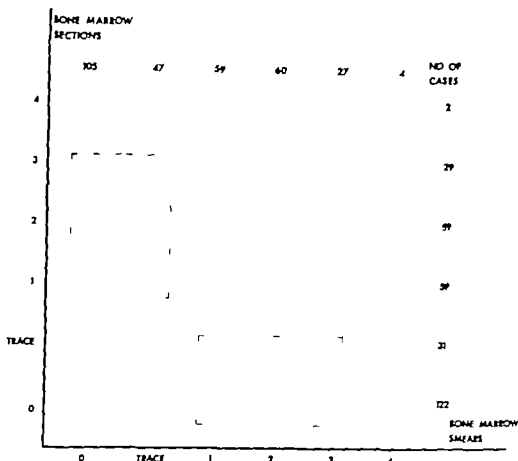


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The group of infectious-toxic anemia with probably concomitant iron deficiency (25 cases) was an ill-defined group. The patients had a moderate or rather severe anemia with a history of blood loss or malnutrition but did not respond to iron therapy

The group treated with parenteral iron (15 cases) comprised cases of chronic iron-deficiency anemia, examined shortly after the parenteral administration of 800—1 500 mg colloidal iron.

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smears respectively. In the group of infection without anemia (24 cases) hemosiderin was absent in four cases examined by sections and in two examined by smear preparations. No tendency to an increase in the amount of hemosiderin can be elicited from this material.

In the group of infectious and hemolytic anemias only five and four respectively patients lacked hemosiderin. On clinical grounds these patients should have well-filled iron stores. Most of these cases had a myeloproliferative disorder and, as was pointed out earlier, it is sometimes difficult to find hemosiderin in such cases (2, 15). This group as a whole shows a tendency to enlarged iron stores as compared with the controls.

The group of infectious toxic anemia clinically appearing to be iron-deficient showed hemosiderin deposits in only three and one case respectively out of 23 examined.

The findings in the last two groups were also in agreement with the clinical assumptions.

On attempting to analyze the discrepancies found, the result was inconclusive. Thus in only seven cases could a confident assessment of the iron stores be done by other means. In five of these cases the examination of histological sections and in two the examination of smear preparations has given results in agreement with these clinical assumptions. In a further ten cases, only a probable clinical prediction could be made. This was in seven cases in agreement with the section and in three with the smear technique.

Conclusions and summary

Three hundred bone marrow aspirates were examined for hemosiderin with the Prussian-blue reaction in fixed histological sections and in smear preparations. There was a good agreement in the results between the two techniques. In 16 % of the cases discrepancies of diagnostic importance were observed. After an analysis of these cases by other means, neither of the methods could with certainty be regarded as superior to the other.

With both methods hemosiderin was absent in all cases of chronic iron-deficiency anemia indicating that the risk of getting false positive results is small.

The absence of hemosiderin must be evaluated in relation to the quantity of marrow examined. This evaluation can be performed with more confidence when fixed sections are used. In practice the same result is obtained with smear preparations provided cellularity is good and thick smears or marrow particles are examined. Several slides should be inspected.

The principal value of hemosiderin examination in hematological disorders is when the result of the determination is positive. In such cases iron deficiency can be excluded. However, the reverse is not true. The absence of hemosiderin in mild anemia does not warrant a conclusion that iron deficiency is its cause. Available iron may still be present in the form of histochemically unstainable ferritin.

For routine hematological practice we recommend the use of thick smear preparations for the examination of hemosiderin, because of its simplicity and because of the ability to evaluate simultaneously bone marrow cytology and the sideroblast count. The latter may render additional information about the state of iron stores.

Acknowledgment

This study was supported by grants from Göteborgs Läkaresällskap and A. B. Leo, Hälsingborg.

Fig 3 Stainable iron in bone marrow sections and smears as related to the clinical diagnosis.

observe that there is a tendency to get a greater number of trace findings in smear preparations as compared with sections. This tendency becomes more evident in fig 2 Here the sum of class 0 and trace in smears consists of 134 cases and in histological sections of 150 cases, but the number of cases with trace is in smears 76 and in sections only 31 Traces of iron, however are difficult to judge and they are found even in frank iron-deficiency anemia.

The reliability of either method was checked in relation to the clinical diagnosis. The clinician is able to predict with certainty the state of iron stores only in two extreme situations. In well established chronic iron-deficiency anemia the iron stores must be empty (2 7 12) Other severe anemias, especially those requiring multiple blood transfusions and without concomitant external blood losses necessarily must have abundant iron stores. The state of the iron stores is not predictable in non anemic subjects, in mild anemia of infection and in acute hemorrhagic anemia.

However in the last mentioned situations a clinical suggestion at least may be made. History of blood losses, determinations of serum iron, and TIBC, sideroblast count and hypochromia may be of value for the clinical evaluation.

Fig 3 shows that hemosiderin was absent in all the 34 clinically diagnosed cases of chronic iron-deficiency anemia. Accordingly it can be concluded that if attention is paid to the sources of error the risk of obtaining false positive results is low with either of the methods used

Seven or five out of 24 men and five or four out of seven women in the control group lacked hemosiderin. This is in accordance with earlier observations made by us and others (8, 15)

In 31 cases of acute hemorrhagic anemia, hemosiderin was present in five persons in sections and in nine persons in

Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

From the Department of Medicine (Head: E. Ask Upmark, M. D.) of the Royal University Hospital of Upsala, Upsala, Sweden

Another Follow-up Study of Children Born of Mothers with Leukemia

By

Erik Ask Upmark

In a previous paper (1) attention was called to the relationships between pregnancy and leukemia. One question of particular importance was the future fate of children born of mothers with leukemia. It turned out that our knowledge on this topic was strictly limited. Only very few cases have been traced for any length of time.

Eight children of mothers with acute leukemia had been traced for 1—10 years (on an average 3.7 years).

Nine children of mothers with chronic myeloid leukemia had been traced for 1—5½ years (average 2 years) outside Sweden.

Three Swedish children of mothers with chronic myeloid leukemia had been traced for 17—25 years (average 20 years).

In the present paper is reported a follow-up of 6 more children born of 3 mothers with leukemia, the follow-up time ranging from 9 years (one case) to 41 years (two cases).

Submitted for publication September 26, 1963.

Material

The material was represented by one Italian, one Norwegian and one Swedish mother. The Italian case is identical with case 5 in the paper of Ruol (7) and it has been possible to trace the twins of this mother only through the kindness and the distinguished efforts of Professor Ruol and the selfless devotion of the priests of the Catholic church. The Norwegian case is identical with case 9 in a paper by Brandt, published in 1923 (2). This paper was overlooked in my previous study and my attention to it was called by Dr. Erland Sartor (9). Only by means of the kind assistance of Professor E. Schjött Rivers (10) and of the gentlemen of the Norwegian clergy has it been possible to trace these children, rather an arduous task after so many years involving war and occupation. The Swedish case was pointed out to me by Dr. Eskil Furst (4) and the family involved examined by myself. To all these colleagues and to the members of the clergy I hereby extend my respectful acknowledgement.

Italian case. Woman aged 34. Chronic myeloid leukemia for 3 years before death. Twins (one son, one daughter) born 1 year before death. The children have been traced

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Italian case Woman aged 34 Chronic myeloid leukemia for 3 years before death. Twins (one son, one daughter) born 1 year before death. The children have been traced

in two orphanages and have been examined by Professor Ruol 10 years after delivery He was so kind as to send me a slide with a blood-sample from each of them. Both children are doing well, their hematology is normal, the boy mentally slightly below average the girl decidedly above average

Norwegian case Woman, in whom chronic myeloid leukemia appeared when she was 34 years of age. When aged 36 delivery of twin-boys. When aged 41 delivery of girl. At birth one of the twin-boys was about half of the weight of the other Whether they were identical twins is not clear the placenta was solitary One of the boys died shortly before the age of 9 from "malignancy The other boy is now 41 and reports himself as entirely healthy as is also his sister now 36 years of age It has so far not been possible to trace the character of the malignancy from which the 9-year-old boy died he had been treated repeatedly in a hospital and leukemia cannot be excluded

Swedish case The mother died from acute myeloid leukemia, when aged 28, shortly after delivery of a girl baby Three years earlier she had delivered a boy who is now 44 and reports himself as entirely healthy This son (who was slightly premature at birth) has at present 4 children, aged 13 (girl) 10 (boy) 8 (girl) and 3 (boy) These children were kindly examined hematologically by Professor Erik Wassén in Gothenburg last summer (11) The hematology was entirely normal and the spleen was not palpable As to the girl born whilst her mother had her leukemia, she is now 41 alive and well. She has been examined by myself personally and except for a slight anemia she was found to be perfectly healthy She was so kind as to bring along her 3 children One boy aged 17 had had the peculiar experience of having had rubella 3 times. His blood was normal, his general condition good and the spleen was definitely not enlarged. The next boy aged 15 had a slight trace of migraine (as had his mother) He was found physically fit in every regard but in his differential count (200 cells) there were 2 % basophils, which is rather on the high side and ought to be watched in the future, as should his slight degree of anemia. The youngest child, a girl aged 11 was likewise found to be a healthy youngster with normal blood. She had the remarkable history of having had 4 fractures of various bones (rib, clavicle tibia) Her sclerae were not blue. Time did not allow a detailed roentgenological examination of her bone system, but there may have been slight torticollis. She as well as her brothers and her mother were of blond, Nordic appearance.

Results

It will be seen that the Swedish case had acute myeloid leukemia (although with considerable enlargement of the spleen) but that her two children and seven grandchildren were normal (one child born whilst the mother had her disease)

The Italian and the Norwegian cases had chronic myeloid leukemia. The Italian children were seen 10 years after delivery of the three Norwegian children one boy was alive 41 years after delivery whilst his twin-brother died at the age of 9 from malignancy and his sister is alive and allegedly healthy 36 years after delivery

The Swedish and the Norwegian observations represent the longest period over which a child from a mother with leukemia on delivery has been traced (41 years each in the Norwegian also a sister aged 36) It goes without saying that the fate of these families should be followed in the future

Discussion

It has rightly been said that the distance in years between the generations of man and the small number of individuals in each generation are handicaps to transmission studies of leukemia in man. That the condition of the chromosomes seems to be of importance is to be seen from the greater incidence of leu

kemia in mothers aged 40 or more and in connection with Down's syndrome, which is also more common in children of mothers of mature age. That exogenous factors may operate is clearly demonstrated by the experiences from Hiroshima and Nagasaki. In mice, Gross has conclusively demonstrated the vertical transmission of leukemia, probably due to a diaplacentary virus. In cattle it is an old experience that one particular bull may rear a stock which, like the bull itself, has leukemia (16 out of 20 cows, sired by a bull with leukemia). In man, the male influence has so far not been investigated. As to the female influence it is obvious that there are instances of congenital (or at any rate very early) leukemia in children, born of healthy mother. To the best of my knowledge the fate of such mothers has not been traced, which certainly ought to be done. It is also obvious from the present study that a mother with leukemia may deliver children who for at least 41 years have remained unafflicted by the disease of their mother. On the other hand it should be emphasized that the material so far available of children born of a mother with leukemia is of very limited size and that every effort should be made to get further our knowledge. The case of Cramblett and coworkers, where leukemia developed in a boy aged 9 months and born of a mother with leukemia, should be remembered and so should the much debated case of Cameron from the 90's. In the present material one boy aged 9 born as one of two twins of a mother with leukemia died from malignancy. Although it has not so far been possible to trace this case in detail it should be remembered that the most common malignant disease at that age is leukemia.

Another factor should be considered as well. An agent (whether virus or not) causing leukemia may well be present for a long time in the body before it manifests itself. A woman from Estland died some 17 years ago in our clinic from acute myeloblastic leukemia. She was then 43, the oldest of 3 sibsters (No. 2 a brother died when young, No. 3 a sister was still alive). She had two sons, aged 3 years and 6 months respectively. The eldest boy died from acute myeloblastic leukemia when 20½ years of age, whereas the younger boy is still alive. In this case no definite conclusions are possible but the case may serve as an example of the possible complexity. The interesting observations on leukemia in monovular twins have recently been reviewed by Ioachim (6) who also summarizes the conflicting evidence about familiar occurrence of leukemia. Although due caution should be exercised, as emphasized by the statisticians, we have a definite impression from the large material collected here in Sweden from three medical departments by Carneskog (3) that there may be a pre-disposition in certain families.

Summary

Six children, born of 3 mothers with leukemia in Italy, Norway and Sweden have been followed up for 9—41 years. One boy died from malignancy when 9, one boy and one girl (twins, Italians) are alive and well after 10 years, one boy (Norwegian) and one girl (Swedish) are alive and well after 41 years and one girl (Norwegian) is so after 36 years. These follow-ups are by far the longest hitherto on record and should be pursued in the future. Pertinent questions are discussed.

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Acta Medica Scandinavica. Vol. 175, fasc. 3, 1964

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Dystrophia Myotonica

A Follow-up Study of a Family with Associated Heart Disease

By

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Dystrophia myotonica or Steinert's disease has been recognized as a clinical entity distinct from myotonia congenita or Thomsen's disease, since the end of the nineteenth century It is a quite rare disease of systemic nature, chronic course, and somewhat unpredictable but probably dominant inheritance.

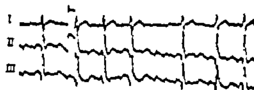
The detailed description of dystrophia myotonica can be found in text-books of neurology (3) but a short review of the many manifestations might be of value.

Most outstanding are the muscular symptoms myotonia and atrophy The earliest symptom is usually myotonia of hands, tongue and feet, followed by atrophy of the same muscle groups. Later comes atrophy of masticatory and sternomastoid muscles.

Cataract, premature frontal baldness, lowered basal metabolic rate, testicular atrophy and mental retardation are other features of the systemic morbid process. The disease usually starts in early adult life.

Since Griffith's report (4) on association of bradycardia and premature heart beats with dystrophia myotonica, many reports on the cardiovascular abnormalities in dystrophia myotonica have appeared. From Scandinavia Ask Upmark (1) has presented eight cases and stressed the frequent finding of brady cardia and prolonged P R interval. Hess Thaysen (9) has given a list of early references and added three cases of his own. Evans (6) described eleven cases with electrocardiographic abnormalities among thirteen cases of dystrophia myotonica. A case presented by Fisch and Evans (7) died from a prolonged attack of auricular flutter Kuhn (10) observed the development of left-bundle branch block in a case followed for ten years. DeWind and Jones (5) collected from the literature all cases of dystrophia myotonica where an ECG was available and added their own six cases. Among a total of 98 cases, 61 had one or more electrocardiographic abnormalities. Cannon (4) reported on the cardiac and pulmonary complications in 11 cases of dystrophia myotonica. Eight of these had ECG abnormalities. In two of his patients the

Submitted for publication October 2, 1963.



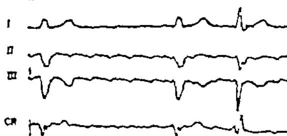


Fig 1

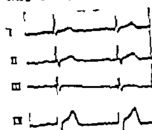
cardiac arrythmia antedated the diagnosis of dystrophia myotonica One case had two Stokes-Adams attacks during an attempt to regularize auricular flutter with digitalis and quinidine. Litchfield (11) reports on a 42 year-old woman with Stokes Adams attacks for seven years Schindler and Forster (13) report on the results of cardiovascular investigations in 24 cases of dystrophia myotonica They range dystrophia myotonica among the cardiomyopathies and coin the expression "dystrophia cordis myotonica They also find that with a long duration of the disease the ECG abnormalities tend to be more frequent and serious Spillane (14) underlines the risk of sudden death in dystrophia myotonica Miller (12) reports one case with serial development of first degree heart block and appearance of complete left bundle branch block after maximal exercise on a treadmill Spurny and Wolf (15) comment on prolonged atrial flutter preceding skeletal muscle involvement by 12 years.

As the natural history and course of rare chronic diseases can be evaluated only by the slow accumulation of case reports in the literature, we felt it of interest to report four cases that were studied in 1944 by one of us (2) three of which have been followed up after 18—20 years. To our knowledge no other study with such a long observation time has been reported.

Case reports

Case 1 The father born 1887 and died 1944 had dystrophia myotonica of increasing severity from the age of 20 He could earn his living as an unskilled worker until death. He abused alcohol and had recurrent attacks of vertigo in the last years, but was otherwise remarkably healthy and had never been admitted to hospital He had no symptoms referable to the heart. In 1942 however he was examined as a part of the family study when the dystrophia myotonica in his children was investigated. An ECG showed a slightly prolonged P R interval (0.22 sec.) but no other abnormality In 1944 he had a bicycle accident and was admitted to hospital in a comatose state He never regained consciousness and died after 5 weeks. During this period he had bradycardia. Only one electrocardiogram was taken. It shows regular rythm, pulse of 50 no identifiable P waves and a normal QRST complex. Post-mortem examination was performed. The heart was dilated and soft, the myocardium pale. The coronary arteries showed only moderate atherosclerosis. The total weight of the heart was 420 g No scarring or fibrosis was found.

Case 2 The elder brother born 1920 has no history of rheumatic fever or diphtheria. In 1942 he noted myotonia in the hands That year he was observed in hospital ECGs showed a constant auricular flutter with irregular block (fig 1) Treatment with digitalis failed to normalize the rhythm, but five days of treatment with quinidine restored sinus rhythm. A P-R interval of 0.22—0.24 sec. was then disclosed. A check a year later revealed unchanged sinus rhythm and P-R interval 0.24 sec.



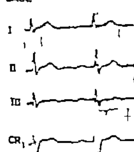


Fig. 2.

In 1937 he was admitted to another hospital after an attack of chest pain and vertigo. The ECGs showed recurrence of the auricular flutter. In the years 1943 to 1957 he had been employed full-time as a docker without experiencing any cardiac troubles.

In 1960 he was again in hospital after frequent attacks of vertigo. The heart rhythm was unstable: sinus bradycardia alternated with auricular flutter and complete heart block. On several occasions ventricular asystolia 5 sec. or less was noted. This time digitalis, quinidine and ephedrine were without effect. He was severely incapacitated by his vertigo attacks. The attacks resulted in fracture of the occipital bones and other less important injuries. The attacks had no resemblance to epileptic or Menière fits.

In 1962 he was seen at a follow-up examination. He was now in an advanced state of dystrophia myotonica with pronounced "facies myopatica", complete atrophy of the sternomastoid muscles, moderate cataract and testicular atrophy. The myotonic reaction was moderate in hands and tongue.

His cardiovascular status was as follows. No subjective or objective signs of heart failure, pulse slow varying from 30 to 45, heart rhythm mainly irregular with short periods of apparent regular bradycardia, no significant murmurs. An ECG showed auricular flutter with complete heart block, very slow idioventricular rhythm and infrequent supraventricular and idioventricular extrasystoles (fig. 1). The QRS duration was 0.15 sec. During the intervening periods of sinus bradycardia right-bundle branch block appeared. The ECG from exercise test showed disappearance of the extrasystoles during maximal work and reappearance at rest. When a phonocardiogram was obtained, he complained suddenly of vertigo. This attack coincided with asystolia for 12 sec. A radiogram of the chest revealed clear lung fields and normal size and configuration of the heart. The EEG showed no epileptogenic activity. As he was seriously ill from the frequent vertigo attacks and in constant danger of prolonged asystolia, and as many trials with drug therapy had failed to restore normal rhythm, artificial pace-making of the heart was started. When the pacemaker functions properly he is absolutely free of vertigo attacks.

Since the start of dystrophia myotonica 20 years ago his heart rhythm showed changes in the form of auricular flutter and then sinus bradycardia with prolonged P R interval. In the last years he has developed a right-bundle branch block and periods of complete heart block have intervened. The coincidence of frequent vertigo attacks and periods of asystolia for as much as 12 sec. is significant.

Case 3. The younger brother born 1921 has dystrophia myotonica with pronounced myotonic symptoms since the age of 20. In 1936 he was in hospital for an illness characterized by fever, chest pains and high ESR and by absence of arthralgiae, erythema and chorea. He had physical signs of pneumonia, confirmed on a chest radiogram.

In 1944 a thorough cardiological examination was performed. The only abnormality found was a minor intraventricular conduction defect on the ECG. The P R interval was



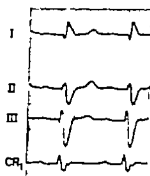


Fig 3

normal and he had a pulse of 55 (fig 2) There were no murmurs. The heart was not enlarged His only sign of dystrophia myotonica at this time was myotonia of the hands.

In 1962 he was reexamined. In spite of very strong myotonia of the hands he has been fully employed as store-house worker He presented the moderately advanced picture of dystrophia myotonica. The myotonia was stronger but the atrophy less than that of his elder brother The significant findings were a regular heart action pulse of 55 no detectable murmurs on auscultation, normal chest X-ray study and on ECG the same small intraventricular conduction defect as 18 years earlier (fig 2)

Although his myotonia is severe and the muscular atrophy in the face and neck has progressed, there were no symptoms referable to the heart. His cardiac status was unchanged after 18 years.

Case 4 The daughter and youngest child born 1926, had an attack of tonsillitis and acute glomerulonephritis in 1929 when the heart was found normal. In 1937 cardiac abnormalities were found incidentally when she was in hospital for gastroenteritis. She had a soft systolic murmur a broad apical impact and a prolonged P R interval of 0.26 sec. No history of rheumatic fever or diphtheria could be elicited She had at this time no subjective complaints and was not in heart failure.

In 1941 she was referred to a cardiological department complaining of palpitations. Auricular flutter with variable block was demonstrated on ECG (fig 3) Treatment with digitalis and quinidine failed and was abandoned At a check in 1942 spontaneous return to sinus rhythm had occurred. The dystrophia myotonica had started during these years and the myotonia was already moderate.

In 1944 a cardiological examination was undertaken On auscultation a soft systolic murmur was noted over the pulmonary area, as well as infrequent premature beats. An ECG showed a P R interval now prolonged to 0 35 sec. and ventricular premature beats interfering with a normal sinus rhythm.

In 1956 she complained of fatigue and low back pain. An ECG showed development of left bundle branch block, QRS duration 0.15 sec. but a now shorter P R interval 0.22 sec. Her working capacity and ECG-reaction to maximal work were normal. Repeated ECG showed that the heart rhythm alternated unpredictable between auricular flutter and sinus rhythm.

In 1962 she was reexamined by us. There were no signs of heart insufficiency the rhythm was regular and the pulse 75. No murmurs could be detected and the heart sounds were feeble An ECG showed sinus rhythm, P R interval of 0.24 sec., left-bundle branch block, with QRS now widened to 0 18 sec., normal ST and T and respiratory arrhythmia (fig 3) Her myotonia is progressing and disabling The muscular atrophy is not advanced to the same extent.

This woman has had intermittent auricular flutter for 21 years. The P R interval has always been prolonged within the range 0.35 to 0.22, but no definite tendency to increase with duration of disease. The QRS complex has widened progressively from less than *0.10* sec. in 1944 to 0 18 in 1962

Discussion

The natural course of dystrophia myotonica is considered to be extremely variable, but few if any reports of cases studied for long periods have appeared in the literature. We have considered it of value to report on this family although it is impossible to make any generalized statements from the follow-up of only one family.

Two cases with Stokes-Adams attacks in dystrophia myotonica are reported in the literature (11, 14). Case 2 in our study has had attacks of vertigo coincident with cardiac arrest for at least 12 sec. Cases 2 and 4 have developed a bundle branch block but case 3 has no significant heart abnormalities.

This study confirms that there is a risk of asystolia in dystrophia myotonica. The heart disease in dystrophia myotonica might more often than suspected have an influence on the prognosis. We emphasize the necessity of considering the heart disease in dystrophia myotonica.

The 3 cases reported in the literature (10, 15) where heart arrhytmia preceded the development of skeletal muscle symptoms might suggest that some, otherwise unexplicable, arrhythmias in young adults could be related to later development of dystrophia myotonica.

Summary

The clinical course of heart disease in dystrophia myotonica in three siblings followed for 18 to 20 years is reported. It is suggested that the heart disease might be of prognostic significance in this condition. The development of asystolia in one case and its successful treatment with artificial pace-making of the heart is reported.

The necessity for cardiological evaluation of cases with dystrophia myotonica is emphasized.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method

V. The Distribution of Insulin-like Activity in Electrophoretically-separated Serum Protein Fractions from Normal Fasting Subjects and the Effect of Ingestion of Glucose

By

Jens Lyngsøe

The investigations so far published on the insulin-like activity (ILA) in electrophoretically-separated serum protein fractions have shown varying results. In one series of studies, only one ILA fraction was demonstrated, associated with the β-γ-globulins (5, 6, 8) while two ILA fractions were found in other series, one fraction associated with albumin-α_1-globulin and the other fraction associated with β-γ-globulins (2, 9, 21). In the present study the electrophoretic distribution of ILA in serum from normal subjects has been examined by means of the rat epididymal fat method. Two ILA fractions were found, associated with the same serum protein fractions as demonstrated in the studies already published. It was shown in a previous study (13) that inactive insulin is present corresponding to both these fractions, and that this insulin is probably attached to protein. The present study investigated the changes in the two ILA fractions following ingestion of glucose.

Submitted for publication August 19, 1963.

Material and methods

Normal subjects were selected from among patients hospitalized for diseases unaccompanied by elevated blood sugar or by glycosuria. They had no symptoms of diabetes mellitus, and examination of the 24-hour urine showed no glycosuria.

These normal subjects were examined fasting between 7 a.m. and 8 a.m., 9–10 hours after their last meal. Some of the patients were then given an aqueous glucose solution to drink (1 g glucose/kg body weight). The patients continued resting in bed and 1/2 hour or 1 hour after the administration of glucose a further sample of blood was withdrawn. The blood was taken from an arm vein applying slight stasis. The serum was prepared at 4° C as in previous studies (12).

The serum insulin-like activity (SILA) and the insulin-like activity (ILA) of the serum protein fractions were determined by a modification of the rat epididymal fat method (11). In some of the assays this method has been modified in order to determine the ILA of 8 unknown samples in one assay. To achieve this, each of the epididymal fat lumps was cut into 5 pieces, 1 piece was incubated in each of the standard insulin solutions, and the remaining 8 pieces from the same rat were

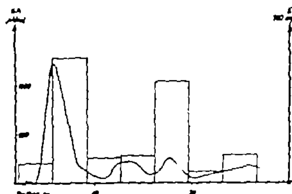


Fig 1 Pat no 1 Fasting serum, assay no 926, lambda 0.31 The columns indicat the ILA of the protein fractions.

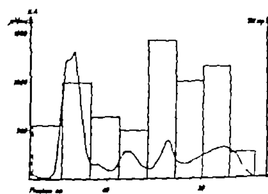


Fig 3. Pat. no 3 Fasting serum, assay no 930, lambda 0.23.

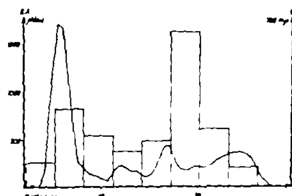


Fig 2. Pat. no 2 Fasting serum, assay no 926, lambda 0.22.

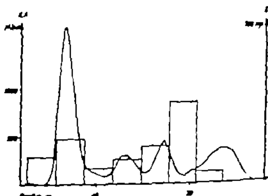


Fig 4 Pat. no 4 Fasting serum, assay no 935, lambda 0.16.

incubated with the 8 unknown samples. Five rats were used for each experiment, and the fat lumps from these were distributed in 50 incubation tubes so that each standard insulin solution and each unknown sample was in cubated with five fat pieces, all of wh ch had differing sites of origin in the fat lumps. By this means, it was possible to compensate for the fact that fat pieces originating from the most peripheral parts of the epididymal fat lumps have a greater glucose oxidation than the pieces which lie more centrally (11 19)

The serum electrophoresis was done in polyvinylchloride blocks as described n previous investigations (13) In the case of the first 8 sera, blocks measuring 0.8 × 7 0 × 38 cm were used to separate 1 ml of serum After electrophoresis for 18 hours at 18–20 mA, each block was cut into 1/2 cm thick sections. Each section was shaken up with 3 ml of 0.9 % NaCl, the solution centrifuged and the protein content of the supernatant was determined (14) so that an ordinary electrophoretic curve was obtained. These small sections were then added together to form larger sections of 5 (or 6) of the 1/2 cm sections. For example, if serum protein is found in 40 sections following electrophoresis these are mixed so that fraction no. 1 contains the first five 1/2 cm sections, carrying the first part of the albumin, fraction no. 2 contains section 6–10 carrying the last part of the albumin and α_1-globulin, fraction no. 3 contains section 11–15 carrying α_2-globulin, and so on. The protein in the fractions so prepared is then eluted by displacement filtration with 0.9 % NaCl.

The electrophoresis of sera nos. 9 to 46 was carried out in a slightly different manner The polyvinylchloride blocks were somewhat shorter 0.8 × 7 0 × 18 cm. The electrophoresis was performed at 16–18 mA for 8 hours.

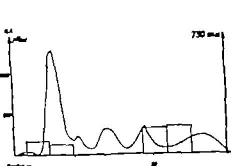
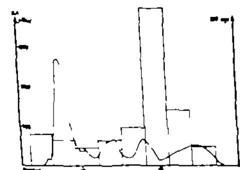
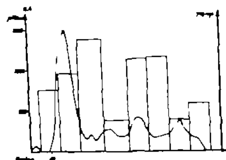

Fig. 5. Pat. no 5. Fasting serum, assay no 939 lambda 0.23.


Fig. 7 Pat. no 7 Serum withdrawn one hour after oral glucose, assay no 941 lambda 0.12.


Fig. 6. Pat. no 6. Serum withdrawn one hour after oral glucose, assay no 940, lambda 0.26.


Fig 8. Pat. no 8. Serum withdrawn one hour after oral glucose, assay no 946, lambda 0.34.

In these experiments the serum proteins were divided into only tw fractions, the block being cut into two parts by cut through the peak of the α_2-globulin. The localization of the fractions was determined in this case with the aid of filter paper strip, as described in an earlier paper (13) The A fraction thus contained albumin, α_1-globulin and the more rapidly moving part of the α_2-globulin, while the B fraction contained the slower component of the α_2-globulin, the β-globulin and the γ-globulin. These fractions were eluted with 0.9 % NaCl by displacement filtration.

After elution, the protein fractions were dialyzed against running water at 20—22° C for 24 hours, then against dextran 20 % (prepared from Macrodex 6 % (Pharmacia A/B Copenhagen) by vacuum evaporation at 60° C. Before use it was sterilized at 120° C for 20 min.) at 18—20° C for 18 hours, and finally against Krebs-Ringer bicarbonate buffer for 8 hours at 4 C. The fractions were then diluted up to the desired volume with Krebs-Ringer bicarbonate buffer The fractions from sera nos. 1—8 were diluted to 12 ml, and from sera nos 9—46 to 20 ml.

The insulin-like activity in the fractions was determined by multiplying the ILA of the diluted fraction by the dilution factor by 12 in sera 1—8 and by 20 in sera 9—46.

All dialyses were made in Visking dialysis tubes of 3.0 cm diameter These tubes were softened for 24 hours in distilled water before use

Results

The first experiments (sera 1—8) were performed in order to elucidate the distribution of the insulin-like activity in the electrophoretically separated serum

Table I Normal subjects

SILA, A and B fraction, determined fasting (I) and after glucose ingestion (II)

Pat. no.	Serum glucose (mg%)	Exp. no.	λ	SILA undiluted serum (μU/ml)		SILA diluted serum (μU/ml)		Exp. no.	λ	ILA A fraction (μU/ml 1/20)		ILA B fraction (μU/ml 1/20)	
				I	II	I	II			I	II	I	II
1 60 minutes after glucose													
9	100–169	1,011	0.20	40	50	80	105	1,017	0.20	3,100	3,200	3,100	4,200
10	103–200	1,012	0.27	25	32	50	60	1,018	0.20	2,300	4,200	7,000	5,300
11	77 123	1,019	0.27	66	85	250	500	1 023	0.26	3,500	2,500	4 700	3,000
12	81 163	1,020	0.33	135	235	270	315	1,024	0.20	7,200	10,800	13,000	12,400
13	80–176	1,025	0.33	140	200	380	330	1,043	0.21	400	760	1 700	2,240
14	93–93	1,026	0.17	112	190	205	230	1,032	0.32	3 700	4 700	3,300	3,100
16	88 100	1,030	0.32	93	105	90	80	1 036	0.29	2,600	4,800	2,500	2,400
21	81–116	1 041	0.32	58	290	62	280	1 049	0.17	1,240	440	1 400	1,040
22	100–187	1,042	0.32	82	180	150	460	1,050	0.12	1,200	2,360	1 440	1,800
23	81–166	1 017	0.31	155	245	195	310	1,053	0.28	340	300	1,900	2,300
24	74–100	1,048	0.32	90	460	235	625	1,054	0.19	360	500	940	760
Mean	87–145			90.7	188.4	178.8	299.5			2,358	3 142	3 725	3,685
2. 30 minutes after glucose													
35	85–160	1,067	0.33	53	120	155	600	1,075	0.25	700	1,320	1,220	800
36	78–120	1 068	0.32	100	160	80	240	1,076	0.34	720	740	1 400	1,740
37	83–123	1,071	0.29	132	180	345	360	1 079	0.16	1,500	1,940	560	520
38	78–111	1,072	0.24	62	132	100	320	1 080	0.15	800	1,320	2,000	2,500
41	80–107	1,077	0 19	245	450	270	625	1,085	0.27	3,200	3,800	2,300	2,440
43	76–108	1 083	0.23	135	205	280	625	1,098	0.32	370	400	760	640
46	83–104	1 088	0.35	70	155	160	210	1 094	0.22	960	380	1,540	1,320
Mean	80–119			113.8	200.3	198.6	425.7			1 179	1 414	1,397	1 423
Mean (fasting value)				99 7 μU/ml		186.5 μU/ml				1,899 μU ml		2,820 μU/ml	

protein fractions The proteins of each serum were divided into 8 fractions and the insulin like activity in these 8 fractions was examined simultaneously in one experiment. This technique provides the optimal conditions for deciding in which fraction ILA is a maximum as it avoids the uncertainty associated with a comparison of ILA determinations made in different assays.

The investigation showed that ILA was present in most of the protein fractions, but it appears from figs 1—8 that the insulin-like activity has a distribution with two maxima, one corresponding to albumin-α -globulin, the A peak, and one corresponding to β-γ-globulins, the B peak. This pattern was found in peripheral venous blood both in fasting normal subjects, and in subjects examined after the ingestion of glucose.

In the subsequent investigation (sera 9—46) therefore, the serum proteins were divided into two fractions, A and B, corresponding to the two maxima of the insulin-like activity Comparative studies

were made of SILA in undiluted and diluted (20 %) serum, and of ILA in the A and B fractions in normal subjects. Values were obtained both for fasting subjects and for subjects who had ingested glucose, 1/2 hour after ingestion in 7 subjects, and 1 hour after ingestion in 11 subjects.

Table 1 shows the results of the investigations. The SILA values in undiluted serum in fasting subjects are seen to vary between 23 and 243 μU/ml with mean value of 99 7 μU/ml. The values in diluted serum vary between 50 and 380 μU/ml, with a mean value of 186.5 μU/ml. At intervals of 30 minutes and one hour after glucose ingestion, higher values are found both in undiluted and in diluted serum.

In fasting normal subjects, the values for ILA in the A fraction vary between 340 and 7,200 μU/ml, with a mean value of 1,899 μU/ml, while in the B fraction the values vary between 560 and 13 000 μU/ml, with mean value of 2,820 μU/ml. The table shows that the SILA values in both undiluted and diluted serum are lower than the ILA values in both the A and B fractions.

Table I also show that fter ingestion of glucose there is a rise in the mean ILA values in the A fraction while ILA in the B fraction does not change. Thirty minutes after the ngestion of glucose, the mean rise in ILA in the A fraction is 235 μU ml, which statistically is not significantly different from 0 ($t = 1\ 419$ $p > 0\ 1$) One hour after the ingestion of glucose the mean rise in ILA in the A fraction is 784 μU/ml which is barely statistically significant ($t = 1.898$ $0\ 1 > p$ $0\ 05$) If all determinations of ILA in the A fraction are considered under one head, the mean rise after ingestion of glucose is 370.5 μU/ml, and this is

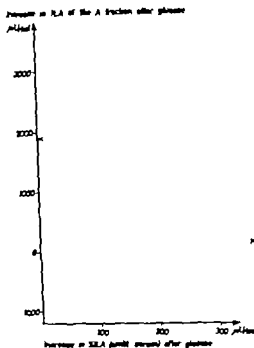


Fig 9 Relationship between the increase in SILA (undiluted serum) and the increase in ILA of the A fraction after glucose administration.

significantly different from 0 ($t = 2\ 167$ $0\ 05 > p > 0\ 02$)

If following glucose ingestion, the mean rise in SILA in undiluted serum and the mean rise in ILA in the A fraction are compared, the rise in SILA is found to be 93 μU/ml and the rise in the ILA of the A fraction is found to be 570.5 μU/ml. The difference between these mean values is hardly statistically significant ($0.1 > p > 0\ 05$ $t = 1\ 780$)

Further table I shows that there is no relationship between SILA in undiluted or in diluted serum, and ILA values in the A or B fraction. Nor is there any dependence between the rise in ILA in the A fraction following glucose ingestion, and the rise in SILA in undiluted serum (fig 9)

Discussion

In the present study of the insulin like activity in electrophoretically separated serum protein fractions from normal subjects all separations showed that the ILA had two *maxima one the A peak* associated with albumin α_1 globulin the other the B peak associated with the *β-γ-globulins. This suggests that serum* contains two insulin fractions. In a number of previously published studies of the same type as the present one only one ILA fraction was demonstrated localized to the β-globulin or to the inter β-γ-globulin fractions, independent of whether ILA was determined by an *in vivo* technique (5) by the rat epididymal fat method (6) or by the rat diaphragm method (8) Other studies by the rat diaphragm method showed two ILA fractions, just as did the present study corresponding to albumin α_1 globulin and to β γ-globulins (2 21) while Gjedde (9) using the rat epididymal fat method finds two ILA fractions localized to *a and γ-globulin respectively The most* disagreement between the studies so far published thus concerns the insulin like *activity of the albumin α_1-globulin frac*tions. It is difficult to compare the studies cited as they use various techniques of electrophoresis *as well* as various techniques for determining ILA Furthermore, the temperature *conditions* during electrophoresis, elution and dialysis *if* it is employed are not described in all the investigations. These conditions are of importance, as a previous study has shown *that* dialysis at *room* temperature can raise the ILA of the serum protein fractions considerably *(10) and that the* greatest increase is noted in the albumin α_1-globulin fraction (13) It is therefore extremely likely that ILA cannot be recorded in albumin-α_1-globulin if the temperature is kept low during electrophoresis and dialysis if any or if a method is employed which has a low sensitivity for demonstrating ILA. In this connection it is of interest to note that all *investigators who were able to demonstrate* ILA in albumin α_1-globulin dialysed the protein fractions at room temperature for *24 hours* or more.

It was shown in a previous study that corresponding to both the ILA fractions, insulin is found which is not activated unless dialysis is performed at room temperature. It was also shown that this activation probably occurs by a release of insulin from an inactive protein-bound form (13) The present study also suggests that insulin is present in an inactive form, associated with both ILA fractions as the ILA values in both albumin-α_1-globulin and β-γ-globulins are considerably higher than in undiluted serum. This does not exclude the possibility that active, non-protein bound insulin can also be found, corresponding to albumin a globulin. *A number of investigators have thus found* that when I [1] insulin is added to serum it migrates together with these protein *fractions* (1 7 17 18) and it is possible that any insulin present in a non-protein bound state in serum migrates in the same way

In a recent communication Anthoniades *(2)* has examined the electrophoretic mobility *of the ILA* absorbed *to a* cation-exchange resin (Dowex 50) He showed that this ILA migrates in association with the β-γ-globulins. This *result suggests that the ILA* found associated with β-γ globulins in the present investigation may be *identical with the* "bound insulin" of Anthoniades In an unpublished study the present author was, however unable to *demonstrate any significant* reduction in ILA (rat epididymal fat method) fol-

owing resin treatment by Anthoniades method, either in albumin-α_1 globulin or in β-γ-globulins. At the present stage, therefore, it is not possible to decide what connection exists between the "bound insulin" of Anthoniades, and the two ILA fractions found in the present study

An immunoelectrophoretic study was recently published in which it was shown that I^{131} insulin is "bound" to α_2 M globulin (9) As this globulin is found in both the A and B fractions of the present study it was conceivable that the presence of this protein is the reason for inactive insulin being found associated with both the fractions. This can hardly be the case, however as earlier studies have shown that inactive insulin is present in protein fractions containing only very small amounts of α_2 M globulin (15) At the present stage, it is not possible to decide whether α_2 M globulin has any significance for the presence of the inactive insulin which has been demonstrated in the study cited and in the present investigation. On the other hand it is reasonable to assume, that the "insulin bearing globulin" recently demonstrated in α_1-globulin by agar electrophoresis, may be of significance for the occurrence of inactive insulin-like activity in the albumin-α -globulin fraction (17)

In the present study an attempt has been made to elucidate the changes in SILA and in ILA in the A fraction (albumin α_1-globulin) and in the B fraction (β-γ-globulins) following ingestion of glucose by normal subjects. In agreement with the results of previous investigations the study showed a rise in SILA both in undiluted and in diluted serum. It was possible to demonstrate a rise in ILA in the A fraction, while ILA in the B fraction remained unchanged. The ILA increase in the A fraction may be due to both increased free insulin and increased bound insulin. As the rise in ILA in the A fraction appears, however to be greater than the rise in SILA in undiluted serum, and as no relationship could be demonstrated between the SILA rise in undiluted serum and the ILA rise in the A fraction an isolated rise of the free insulin in the serum seems to be excluded. The results obtained can be explained, however by a simultaneous rise in free and bound insulin, or — less likely — by a rise only of the bound insulin.

The studies by Samaan et al (20) have been carried out with a method which cannot be directly compared with the present one. It is probable, however that the SILA not inhibited by anti-insulin, the so-called atypical ILA" indicates protein-bound insulin in serum. It seems puzzling therefore, that *no change in* atypical ILA could be demonstrated following glucose ingestion in normal subjects.

A few investigations, in which an insulin extraction method has been used, have shown ILA values of the same magnitude as the present study Band and Bornstein (4) using an in vivo method for the insulin assay of extracts from sera, found ILA values between 800 and 1 150 μU/ml in 3 fasting normal persons. In extracts from serum from normal persons on normal diet higher values were found. Pellegrini et al. (16) found fasting values between 2 000 and 30,000 μU/ml in 11 normal persons using the same extraction technique but with an immunological method for the determination of ILA. The very high ILA values found in these studies are in accordance with the results obtained in the present study Large amounts of insulin seem to be present in the serum, which is liberated by extrac

tion procedures or during electrophoresis and dialysis. They also support the findings reported here that ingestion of glucose leads to a rise in ILA which cannot be due solely to a rise in free insulin.

The biological significance of the two forms of inactive insulin is unknown. It seems reasonable to assume that insulin which is inactive on rat epididymal fat is also inactive *in vivo* and that only non protein bound insulin is active *in vivo*. This assumption finds support in some studies which show that severe diabetic symptoms can be elicited in a variety of animal species by the injection of anti insulin, as it is presumably only the non protein bound insulin that can be inhibited by anti insulin (3 15). On the other hand the above hypothesis does not explain why all SILA studies using *in vivo* methods show values higher than those measured by immunochemical methods.

Summary

The present study of insulin like activity (ILA) in electrophoretically separated serum protein fractions has demonstrated the presence of two ILA maxima the first corresponding to albumin-α_1 globulin the A peak the second corresponding to β-γ-globulins, the B peak.

Glucose ingestion by normal subjects was followed by a rise in ILA in albumin α_1-globulin while ILA in β-γ-globulins remained unchanged. The study suggest that glucose ingestion is followed by a rise in the presumably protein bound insulin associated with albumin α globulin

Acknowledgements

Supported by Statens almindelige Videnskabsfond Denmark

The insulin was supplied through the courtesy of Dr H. C. Hagedorn M. D Nordisk Insulin Laboratorium, Denmark.

I wish to express my gratitude to Mrs. Tove Dreyer Sorensen for skilled technical assistance.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Adrenal Hemorrhage and Necrosis in the Adult

A Clinicopathological Study of 23 Cases

By

A. Botteri and S. R. Orell

Adrenal hemorrhage is most often associated with Waterhouse Friedrichsen's (W.F.) syndrome. Clinically this is characterized by a fulminant course, shock, hyperpyrexia, extensive purpura and hypoglycemia. The syndrome is commonest in children though it can also occur in adults. It is now thought to originate from septicemia, usually meningococcal septicemia.

In recent years increasing numbers of cases have been described of adrenal hemorrhage in adults without any connection with septicemia. In such cases the clinical picture is less characteristic and yet it still has several aspects in common with the W.F. syndrome. We recently encountered two cases of this type within a short period and consequently we examined all the post mortem records at the Södersjukhuset for the last twelve years in order to study the types and frequency of adrenal lesions in the adult.

Submitted for publication August 20, 1963.

Material and methods

The primary material consisted of 10,832 clinical post-mortem examinations of adults (over 20 years old) carried out at the Soder sjukhuset between 1950 and 1962. On an average 95 % of the patients who die at this hospital are subjected to post-mortem examination. Histological examination of the adrenals has not been carried out on all cases but in all the cases of macroscopically visible adrenal hemorrhage histological sections of the adrenals were available. In some cases other endocrine organs particularly the pituitary were also examined histologically.

After a survey of the records, 19 cases were found to have macroscopically visible adrenal hemorrhage and infarcts without relation to tumors. In another 4 cases minor adrenal bleedings became visible through microscopic examinations carried out on account of uncertain diagnoses. The material thus comprises total of 23 cases of adrenal hemorrhage and infarction in adults.

These cases can be divided up into three well-defined groups on basis of the pathological-anatomical picture. The largest group, group I comprising 11 cases, is characterized by mainly hemorrhagic infarction of one or

Table I Group I

Case	Age Sex	Primary disease	Other clinical facts	Symptoms	Time to death	Pathology of adrenals	
						Macrosc.	Microsc.
1	56♀	Septicemia	Blood culture: β-streptococci	Hyperpyrexia Hypotension	Short	Almost total infarction of both	Thrombosis of medullary veins
2	77♀	Cerebral arteriosclerosis and softening	Suspected cholecystitis	Abdominal pain + palpable resistance. Hypotension	20 hrs	Right: total infarction. Left: slight hypoplasia	Right: venous thrombosis. Left: tubular cortical degeneration (Rich)
3	67♂	Chron. bronchitis + emphysema	Bronchopneumonia	Hyperpyrexia Hypotension	Short	Right total infarction. Left partial infarction	Bilat. venous thrombosis. Tubular degeneration in cortical remains
4	82♂	Pemphigus. Hypertension + general arteriosclerosis	Bronchopneumonia. 6 months cortison treatment prior to death	Acute heart failure	4 days	Right: total infarction. Left only incipient infarction	Bilat. venous thrombosis. Fibrinoid degeneration of a few arterioli
5	81♂	Cardiosclerosis with acute myocardial infarction	Heparin + dicumarol treatment	Hypotension	Short	Almost total bilateral infarction	Bilat. venous thrombosis. Fibrinoid degen. of a few arterioli
6	71♀	General arteriosclerosis with cerebral softening	Asthmatic bronchitis for 20 years	Hyperpyrexia Hypotension	Short	Extensive bilateral infarction	Bilat. venous thrombosis. Fibrinoid degeneration of a few arteriol
7	75♀	Hypertension + coronary insufficiency	Heparin + dicumarol treatment	Hypotension	7 hrs	Right extensive infarction. Left normal	Right: venous thrombosis. Left no change
8	66♂	Cardiosclerosis + asthmatic bronchitis for 20 years	Minor pulmonary emboli. Venous thrombosis	Hypotension	24 hrs	Right total infarction. Left incipient infarction	Bilat. venous thrombosis. Fibrinoid degen. of few arterioli
9	66♂	Stone in common bile duct with biliary cirrhosis	Choledochotomy without compl. Norm. pr thromb.time	Hypotension	3 days	Right: infarction of ½ adrenal. Left normal	Right venous thrombosis. Left normal
10	54♂	Diabetes mellitus + insulin shock	Bronchopneumonia + cystopyelonephritis	Hypotension Hyperkalaemia Uremia	5 days	Extensive bilateral infarction	Bilat. venous thrombosis
11	36♀	Normal pregnancy without toxicosis	Ext. bleed. after deliv. Blood fib. 0.06 mg%	Agitation dizziness Hypotension	24 hrs	Extensive bilateral infarction	Bilat. venous thrombosis

Table II Group II

Case	Age Sex	Primary disease	Other clinical facts	Symptoms	Time to death	Pathology of adrenals
12	66 ♂	Gastric cancer without metastases	Gastric resection performed without complications	Hyperpyrexia Hypotension	Short	Left: massive medullary bleeding + small cortical necroses. No vascular changes. Right: normal
13	66 ♀	Thrombocytopenia. Alternating ACTH and cortisone treatment for two years	Splenectomy. No postoperative complications	Hyperpyrexia Hypotension	24 hrs	Pronounced adrenal hypoplasia with multiple small cortical bleedings and necroses
14	73 ♀	General arteriosclerosis	Bronchopneumonia. Psoriasis	Hyperpyrexia Hypotension	A few hours	Multiple cortical bleedings bilaterally macroscop. visibl in lef
15	40 ○	Chronic duodenal ulcer. Gastric resection performed	Postoperatively administration of ACTH and cortisone. Bronchopneumonia	Anorexia. Hypotension. Increasing blood NPN	3 weeks	Bilateral adrenal hyperplasia. Vasculitis and multiple small cortical necroses in right
16	60 ♀	Periarthrosis humeroscapularis, mobilized under general anesthesia	No signs of adrenal cortical insufficiency previously	Sudden dizziness	Short	Pronounced adrenal hypoplasia. Multiple cortical bleedings bilaterally
17	64 ♀	General arteriosclerosis + cholelithiasis. Subjected to cholecystectomy	Postop. femoral thrombosis and pulmonary embolism	Hyperpyrexia Hypotension	5 days	Scattered small cortical bleedings bilaterally
18	21 ♀	Pneumonia + septicemia	Culture from tonsils and lungs: staph. aureus	Hyperpyrexia Hypotension	A few hours	Multiple macroscop. visible cortical bleedings bilaterally + tubular degeneration (Fich)
19	44 ♂	Acute intestinal obstruction suspected. Laparotomy: nothing pathological	Chronic alcoholism, otherwise healthy	Acute abdominal pain. Agitation. Hyperpyrexia. Hypotension	24 hrs	Multiple macroscop. visible cortical bleedings and tubular degeneration (Rich) bilaterally

both of the adrenals. Group II comprises 8 cases with multiple minor cortical bleedings, occasionally combined with small necroses. The 4 cases with only microscopically visible lesions are included in this group. Group III consists of 4 cases with old encapsulated lesions, all unilateral. The cases are listed with their essential clinical and pathological data in tables I–III. Certain clinical and pathological aspects are dealt with in more detail below

Table III Group III

Case	Age Sex	Primary disease	Cause of death	History of adrenal disease	Pathology of adrenals
20	51♀	Chronic glomerulonephritis	Uremia	Not known	Left old encapsulated hematoma the size of a hazel nut. Right normal
21	69♀	General arteriosclerosis	Cerebral softening	4 months prior to death acute abdominal pain + palpable abdominal resistance + dislocation of left kidney at urography	Left: old encapsulated hematoma the size of a mandarine. Right: normal
22	50♂	Polycythemia Hypertension	Cerebral softening	Not known	Right old hematoma the size of a plum. Left: normal
23	57♀	Hypertension	Cerebral hemorrhage	5 years before death acute abdominal pain with palpable resistance. Urography: dislocation of left kidney	Left old partly calcified hematoma the size of hazel nut. Right normal

CLINICAL OBSERVATIONS

I Clinical background and primary diseases

Age and sex 10 male patients, 13 female patients. The youngest patient was 21 the oldest 82

Septicemia. Only two cases had manifest septicemia, the primary focus on both occasions being the pharynx and the upper respiratory tract. In case 1 bacteriological examination of blood lymph nodes and tonsils after death revealed beta streptococci in pure culture In case 18 abundant staphylococcus aureus was cultivated from the tonsils before death and from the lungs at the post mortem.

Acute infection without any signs of septicemia occurred in several cases. Four cases (3 4 14 15) had bronchopneumonia to such an extent that it can be assumed to have affected the course of the disease One case (10) had both bronchopneumonia and acute cystopyelonephritis.

Surgery Six patients died in close connection with an operation Two cases (12, 15) were subjected to gastric resection and two (9 17) to operations on the bile ducts. In one case (13) a splenectomy was performed and in another (16) the shoulder joint was mobilized under general anesthesia.

Pregnancy In one case (11) symptoms of adrenal damage appeared about one hour after delivery The patient had shown no signs of toxemia and the delivery was normal. After delivery the patient bled profusely and plasma fibrinogen was found to be very low 0 06 mg

Anti-coagulant therapy In two cases adrenal infarction appeared during anti-coagulant treatment for coronary insufficiency In case 7 heparin was administered for four days and dicumarol for the following five days until death The protrombin time (Quick) was around 70 during the last few days. In case 5 heparin was given for the first day and dicumarol for the following fifteen days. During the entire course of the treatment the protrombin time (Quick) was between 44 and 63 None of the patients showed clinical signs of bleeding

ACTH therapy Case 13 had therapy-resistant thrombocytopenia with varying thrombocyte counts, the lowest being 10,000. The patient was treated periodically for two years with alternating ACTH and cortisone. She died 48 hours after splenectomy without undue bleeding The final thrombocyte count

was 20,000. The post mortem revealed pronounced hypoplasia of both adrenals with small cortical bleedings and necroses.

Case 13 suffered from chronic duodenal ulcer with pyloric stenosis and was subjected to gastric resection. A week after the operation 40 I.U. ACTH was administered daily for ten days, followed by ten daily doses of 100 mg cortisone. The blood non-protein nitrogen nevertheless rose steadily and was over 300 mg% when the patient died. The post mortem revealed hyperplastic adrenals with multiple cortical necroses unilaterally. The kidneys were normal.

Asthmatic bronchitis. Three cases (3, 6, 8) had suffered from severe asthmatic bronchitis for 10 to 25 years. All three patients had been admitted to hospital on several occasions for upper respiratory infections. None of them had been treated with ACTH or cortisone.

Hypertension. Four patients (4, 20, 22, 23) had previously known hypertension with fixed diastolic blood pressure of over 130 mm Hg. Two patients (8, 17) had moderate, labile hypertension.

Other factors. Case 10, a known insulin addict, was admitted after prolonged insulin shock and was put into respirator. His diabetes was extremely labile. The blood sugar showed a tendency to stabilize following administration of hydrocortisone. On the fifth day there was sudden fall in blood pressure. The patient died ten days after admission and then had hemorrhagic infarction of both adrenals and advanced bronchopneumonia and cystopyelonephritis.

Case 4 had pemphigus accompanied by serious hypertension and arteriosclerosis. Case 22 had polycythemia with marked tendency to vascular thrombosis. Cases 13 and 16 had pronounced adrenal hypoplasia without earlier symptoms of adrenal insufficiency. Both patients died after surgery and both revealed small cortical bleedings.

II. Symptoms

Abdominal pain localized to the epigastrium occurred in four cases (2, 19, 21, 23). In each case the pain was of such nature that acute abdominal disease was suspected. In case 21 the pains were uncharacteristic. Case 2 was regarded as cholecystitis and case 23 as acute pancreatitis. Case 19 underwent laparotomy for suspected intestinal obstruction.

Palpable resistance in the upper abdominal region corresponding to the adrenal hematoma was found in three cases. In case 2 a resistance was palpated below the right arcus and this was interpreted as a distended gall bladder. In case 21 i.v. urography and arteriography was carried out, which revealed a soft tissue mass which displaced the left kidney caudally. Urography was likewise carried out in case 23, revealing a dislocation of the left kidney. Roentgen examination also revealed a dislocation of the stomach and the colon, though to a lesser degree. Renewed roentgen examination some two weeks later showed a manifest reduction of the soft tissue mass.

Blood pressure fall. There is no record of the blood pressure in three cases (16, 20, 22). In all the other cases, however, there was a fall in blood pressure, mostly down to shock level. In those cases where the blood pressure did not fall to shock level hypotension was prolonged.

Hyperpyrexia occurred in 9 cases. In 5 of these cases the increase in temperature was explainable by manifest respiratory or urinary tract infection. In the other four cases there was no evidence of infection.

Psychic symptoms. Three patients (11, 16, 19) showed extreme agitation and dizziness in connection with the blood pressure fall.

Laboratory investigations. Two cases (10, 13) revealed manifest hyperkalemia and high blood non-protein nitrogen. In the other cases there was no satisfactory record of blood electrolytes, etc.

Skin changes. Neither purpura nor exanthema was noted in any of the cases.

Duration of symptoms (reckoned from the blood pressure fall). Nine patients died rapidly within few hours. Five patients died after about 24 hours, four within five days and one after about three weeks. The four patients in group III survived the adrenal lesion without treatment and they died long time afterwards from unrelated diseases. From the histories of cases 21 and 23 (pains, palpable resistance, roentgen examinations, etc.) it was possible to establish the time when the bleeding occurred, i.e. 4 months and 5 years respectively before death.

III. Therapy

Eight patients from groups I and II with unilateral and bilateral adrenal bleeding/ne-

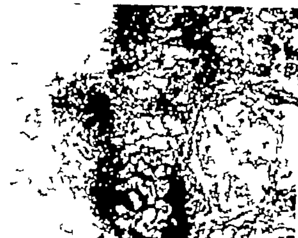


Fig 1 Hemorrhagic infarction of adrenal. To the left cortical tissue showing coagulation necrosis. To the right large medullary vein occluded by fresh thrombus. Hematoxylin-eosin. × 40.



Fig 2. Hemorrhagic infarction of adrenal Coagulation necrosis of cortex with only slight bleeding Fresh thrombosis of large medullary vein Hematoxylin-eosin × 40

farction were given anti-shock treatment which had only a transitory effect on the blood pressure. Four of these were treated with both hydrocortisone and noradrenalin besides saline-glucose or whole-blood infusions. Cases 10 and 11 who had complete infarction of both adrenals received 100 mg×4 hydrocortisone every 24 hours. They survived 72 hours and 24 hours respectively Up to 32 mg daily of noradrenalin was given.

PATHOLOGY

Group I These cases were characterized by acute hemorrhagic infarction of one or both adrenals. The bleeding was often limited to the medulla and the inner part of the cortex, while the outer part of the cortex revealed coagulation necrosis without much bleeding (figs 1 and 2)

In eight cases the infarction was bilateral, equally advanced on both sides in five of these. In the other three the infarction was almost total on the right side but only limited or just beginning on the left. In three cases only the right adrenal was affected.

In all eleven cases there were fresh thrombi in the major medullary veins (figs 1 and 2) It is remarkable that in two cases one adrenal was totally infarcted while the other showed only the beginning of necrosis without bleeding despite the fact that there were fully developed thrombi in the medullary veins on both sides. In a few cases there was also thrombotization of the small veins in the fatty tissue around the adrenals. In seven cases there was a slight inflammatory reaction in the vein walls at the site of the thrombi while in the others there was no reaction at all. Case 8 had femoral thrombosis with pulmonary embolism, otherwise there were no cases of thrombosis in other organs.

In four cases the small arteries in the fatty tissue around the adrenals revealed fibrinoid degeneration of the vessel walls with thrombosis (fig 3) These changes were probably secondary to the bleeding and necrosis which extended from the adrenals into the surrounding tissues. Only in one patient (case 8) who was suffering from bronchial asthma, were these changes interpreted as a genuine vasculitis of the allergic type. There were no significant arteriosclerotic vascular lesions in any of the cases.

Group II These cases had multiple small bleedings in the adrenal cortex (fig 4) Cases 12 and 13 had both bleedings and small cortical necroses, case 15 only small necroses. In the latter case some small cortical vessels also revealed vasculitis. Otherwise there were no vascular lesions and no medullary vein thrombosis in these cases. The adrenal lesions were bilateral in all cases except two. Case 12 had massive medullary bleeding and small cortical necroses in the left adrenal, while in

the right one there were no changes. Case 15 had only unilateral cortical necroses.

In cases 13 and 16 the adrenals were extremely hypoplastic with an irregular cortical structure. Thus the adrenals were damaged before the actual bleeding took place.

Group III These cases had old encapsulated lesions in one of the adrenals three cases the left, one case the right. Macroscopically these were seen as cysts filled with red-brown, muddy mass. The size varied from that of hazel nut to mandarine. Microscopic examination revealed an amorphous mass surrounded by thick capsule of connective tissue containing inflammatory cells, iron pigments and cortical tissue remnants. In case 23 the capsule was extensively calcified. There was no evidence of either venous thrombosis or other vascular changes.

Tubular degeneration. This characteristic change in the outer part of the adrenal cortex (zona fasciculata) which has been described by Rich in connection with severe acute infections with peripheral circulatory collapse (31) was observed in two cases in group I (2, 3) and two cases in group II (18, 19) (fig. 5) In case 3 there was Rich degeneration in the cortical remnants of the infarcted adrenals. In case 2 the right adrenal was infarcted while the left one revealed Rich degeneration without any other changes. In cases 18 and 19 there was Rich degeneration simultaneously with small cortical bleedings.

Other endocrine organs. The pituitary was examined in six cases, three in group I (4 10 11) and three in group II (13, 16, 17) Only in case 16, where there was pronounced adrenal cortical atrophy did the pituitary show any pathological changes Crooke degeneration of the basophilic cells in the frontal lobe. I five cases other endocrine organs were examined without any pathological findings.

Discussion

According to the literature adrenal bleeding seldom occurs in adults and then briefly in connection with septicemia. In 1953 Berte (4) collected 22 cases from the literature and described



Fig 3. Hemorrhagic infarction of adrenal. T the left necrotic adrenal cortex. T the right small vessels in the surrounding fat tissue showing fibrinoid degeneration and thrombosis. Hematoxylin-eosin. × 40.



Fig. 4 Adrenal cortex showing multiple small bleedings and tubular degeneration of Rich. Hematoxylin-eosin. × 40.



Fig. 5. Tubular degeneration of Rich in outer adrenal cortex. Hematoxylin-eosin. × 100.

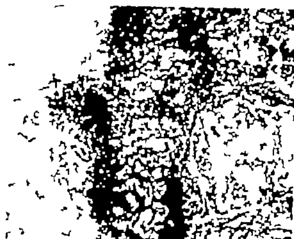


Fig 1 Hemorrhagic infarction of adrenal To the left cortical tissue showing coagulation necrosis. To the right large medullary vein occluded by fresh thrombus. Hematoxylin-eosin. × 40



Fig 2. Hemorrhagic infarction of adrenal Coagulation necrosis of cortex with only slight bleeding Fresh thrombosis f large medullary vein. Hematoxylin-eosin × 40

farction were given anti-shock treatment which had only a transitory effect on the blood pressure. Four of these were treated with both hydrocortisone and noradrenalin besides saline-glucose or whole-blood infusions. Cases 10 and 11 who had complete infarction of both adrenals received 100 mg×4 hydrocortisone every 24 hours. They survived 72 hours and 24 hours respectively Up to 32 mg daily of noradrenalin was given.

PATHOLOGY

Group I These cases were characterized by acute hemorrhagic infarction of one or both adrenals. The bleeding was often limited to the medulla and the inner part of the cortex, while the outer part of the cortex revealed coagulation necrosis without much bleeding (figs 1 and 2)

In eight cases the infarction was bilateral, equally advanced on both sides in five of these. In the other three the infarction was almost total on the right side but only limited or just beginning on the left. In three cases only the right adrenal was affected.

In all eleven cases there were fresh thrombi in the major medullary veins (figs 1 and 2). It is remarkable that in two cases one adrenal was totally infarcted while the other showed only the beginning of necrosis without bleeding despite the fact that there were fully developed thrombi in the medullary veins on both sides. In a few cases there was also thrombotization of the small veins in the fatty tissue around the adrenals. In seven cases there was a slight inflammatory reaction in the vein walls at the site of the thrombi while in the others there was no reaction at all. Case 8 had femoral thrombosis with pulmonary embolism, otherwise there were no cases of thrombosis in other organs.

In four cases the small arteries in the fatty tissue around the adrenals revealed fibrinoid degeneration of the vessel walls with thrombosis (fig 3) These changes were probably secondary to the bleeding and necrosis which extended from the adrenals into the surrounding tissues. Only in one patient (case 8) who was suffering from bronchial asthma, were these changes interpreted as a genuine vasculitis of the allergic type. There were no significant arteriosclerotic vascular lesions in any of the cases.

Group II These cases had multiple small bleedings in the adrenal cortex (fig 4) Cases 12 and 13 had both bleedings and small cortical necroses, case 15 only small necroses. In the latter case some small cortical vessels also revealed vasculitis. Otherwise there were no vascular lesions and no medullary vein thrombosis in these cases. The adrenal lesions were bilateral in all cases except two. Case 12 had massive medullary bleeding and small cortical necroses in the left adrenal, while in

the right one there were no changes. Case 15 had only unilateral cortical necroses.

I cases 13 and 16 the adrenals were extremely hypoplastic with an irregular cortical structure. Thus the adrenals were damaged before the actual bleeding took place.

Group III. These cases had old encapsulated lesions in one of the adrenals three cases the left, one case the right. Macroscopically these were seen as cysts filled with red-brown, muddy mass. The size varied from that of hazel nut to mandarine. Microscopic examination revealed an amorphous mass surrounded by a thick capsule of connective tissue containing inflammatory cells, iron pigments and cortical tissue remnants. In case 23 the capsule was extensively calcified. There was no evidence of either venous thrombosis or other vascular changes.

Tubular degeneration. This characteristic change in the outer part of the adrenal cortex (zona fasciculata) which has been described by Rich in connection with severe acute infections with peripheral circulatory collapse (31) was observed in tw cases in group I (2, 3) and tw cases in group II (18, 19) (fig. 5) In case 3 there was Rich degeneration in the cortical remnants of the infarcted adrenals. In case 2 the right adrenal was infarcted while the left one revealed Rich degeneration without any other changes. In cases 18 and 19 there was Rich degeneration simultaneously with small cortical bleedings.

Other endocrine organs. The pituitary was examined in six cases, three in group I (4, 10, 11) and three in group II (13, 16, 17) Only in case 16, where there was pronounced adrenal cortical atrophy did the pituitary show any pathological changes Crooke degeneration of the basophilic cells in the frontal lobe In five cases other endocrine organs were examined without any pathological findings.

Discussion

According t the literature adrenal bleeding seldom occurs in adults and then chiefly in connection with septicemia. In 1933 Berte (4) collected 22 cases from the literature and described



Fig. 3. Hemorrhagic infarction of adrenal. T the left necrotic adrenal cortex. T the right small vessels in the surrounding fat tissue showing fibrinoid degeneration and thrombosis. Hematoxylin-eosin. × 40.



Fig. 4. Adrenal cortex showing multiple small bleedings and tubular degeneration of Rich. Hematoxylin-eosin. × 40.



Fig. 5. Tubular degeneration of Rich in outer adrenal cortex. Hematoxylin-eosin. × 100.

two cases of his own. Since then some 40 cases have been reported. Bleedings in connection with septicemia or tumors have not been included in this figure.

These case reports give no precise idea of the frequency of adrenal hemorrhage in the adult. Our material has returned a frequency figure of 0.14 % (15 cases out of 10 852 post mortems) for "massive adrenal infarction or hemorrhage" (groups I and III). The eight cases in group II have not been included, since this group would certainly have been much larger if the adrenals had been consistently examined histologically. Consistent histological examinations of the adrenals in routine post mortems have been carried out by Mitchell and Angrist (26) and by Plaut (28a) and these have shown considerably higher frequency figures (0.6 % and 9 respectively). In these materials however there was but poor correlation with the clinical picture.

Adrenal bleeding and necrosis have been described in connection not only with septicemia and severe acute infections but also with numerous other conditions. These include pregnancy and delivery, hypertension, burns, trauma during convulsions in epilepsy or during electro-convulsive therapy, pemphigus, surgical operations and chronic or asthmatic bronchitis (1, 2, 7, 8, 12, 13, 14, 15, 18, 20, 21, 25, 30).

In recent literature nine cases have been described of adrenal hemorrhage during anti-coagulant therapy (5, 16, 17, 24, 27). Two such cases are included in our material. It is interesting to note that both of these cases showed infarction of the adrenals with thrombi in the medullary veins. Only in one of the previously described anti-coagulant treatment cases was medullary vein thrombosis observed.

Recently the connection between the administration of ACTH and adrenal damage has been the subject of great interest. Several cases have been described of adrenal injuries following ACTH treatment (11, 15, 27, 36). Deaths have been reported following ACTH tests on patients with adrenal cortical hypoplasia (33). Our material includes two cases where the patient was treated with standard doses of ACTH. Both cases had microscopic bleedings and/or necroses in the adrenal cortex. Wilbur and Rich (35) have shown through animal experiments that adrenal injuries in the form of tubular degeneration can result from large doses of ACTH. These observations indicate that adrenal injury can be caused by ACTH treatment. It seems likewise possible that increased endogenous ACTH secretion in certain conditions may produce adrenal injury.

Other hormonal factors may also be of certain significance. Barta et al. (3) have produced adrenal bleeding in animals by provoking insulin shock and simultaneously administering cortisone. Our case 10 may be an example of such a mechanism. The patient was a known insulin addict and was admitted to the hospital in deep insulin shock. He received large doses of hydrocortisone. He died after five days treatment and was then found to have massive infarction of both adrenals.

As has already been pointed out, our material contains two different main groups of adrenal injury: hemorrhagic infarction and small bleedings. Berte (4) previously made a similar distinction. Many authors however do not make a clear distinction between hemorrhagic infarction and simple bleeding. The pathogenesis is probably not identical for these two types of adrenal lesion.

In our opinion hemorrhagic infarction of the adrenals is secondary to thrombosis of the medullary veins. In all the cases of this type we have found such thrombosis. In two cases fully developed thrombi were observed in the major medullary veins in both adrenals but there was only very limited cortical necrosis without bleeding in the one while there was total infarction in the other These findings support our claim that the thrombosis is causative. Meanwhile the etiology of the thrombosis cannot be discovered. Only in two cases was there any possibility of a general tendency to vascular thrombosis. Hypothetically it is conceivable that in strongly increased adrenal cortical activity coagulation-promoting or intima-injurious metabolites may appear in the adrenal venous blood

On the other hand it would seem most probable that the multiple, small cortical bleedings and necroses are caused by capillary damage. Tubular degeneration of the Rich type occurred in both groups, a fact which may imply a common etiological factor

The clinical picture can be dominated by acute abdominal pain which may be of such a nature as to call for laparotomy When there is unilateral massive hemorrhage infarction it is possible to find a palpable resistance laterally in the epigastrium and, by urography a soft tissue mass at the upper pole of the corresponding kidney But abdominal pains may also occur in cases with multiple small bleedings which naturally cannot be verified either by palpation or by roentgenography Our case 19 underwent laparotomy for suspected intestinal obstruction but the findings at the operation were negative In this case the post mortem revealed minor bleedings in both adrenals No other explanation could be found for the abdominal pains. Hyperpyrexia which occurs during the final stage, is of course, a very unspecific symptom, but it can be striking in cases without simultaneous signs of infection. Anorexia, hypoglycemia, etc. may also occur (17 27) Hyperkalemia seems to be a very early symptom. The blood non-protein nitrogen has a tendency to rise continuously to high values, depending upon how long the patient survives.

In the case of unilateral adrenal hemorrhage/infarction the prognosis is not hopeless. In the literature eight cases are described where the patient survived after extirpation of the diseased adrenal (9 10 21 22, 25 28, 29) All of these cases suffered acute abdominal pains and in six of them urography revealed a depression of one of the kidneys. In none of the cases was the adrenal injury diagnosed pre-operatively Our four cases in group III survived the adrenal injury without treatment and died considerably later from unrelated diseases.

Summary

The authors report on 15 cases of massive hemorrhage and/or infarction of one or both adrenals in the adult, two of the cases being personal observations In eleven of the cases adrenal infarction was caused, in the authors' opinion by thrombosis of large medullary veins Such thrombosis was observed in all these cases, including two which were subjected to anti-coagulant therapy and one with fibrinolysis Eight cases are also reported with multiple small cortical bleedings and necroses

The clinical picture is quite consistent irrespective of whether the adrenal damage is unilateral or bilateral and of whether there is massive hemorrhage/in

farction or multiple small bleedings Unilateral damage can lead to death even though the other adrenal may be intact Blood pressure fall is a constant symptom and appears early If the blood pressure does not fall to shock level the hypotension is persistent Hyperkalemia hypoglycemia and dizziness appear at an early stage, rising blood non protein nitrogen and hyperpyrexia at a later stage.

Abdominal pains can occur in connection with massive hemorrhage/infarction or multiple small bleedings The pains can be similar to pancreatitis cholecystitis ileus etc. Large adrenal hematomas are sometimes palpable as a resistance laterally in the epigastrium and i v urography then discloses a depression of the kidney

While the majority of the patients die quickly some can survive for days or weeks Four cases with unilateral massive hemorrhage survived without treatment and died long afterwards from unrelated diseases. Our observations indicate that in massive hemorrhage or infarction hydrocortisone alone, even in doses of 400 mg daily cannot maintain the electrolyte balance Thus mineral corticoid should also be administered

The connection between adrenal injury and exogenous and endogenous ACTH stimulation anti-coagulant therapy etc, is also discussed

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Fat Concentration in Faeces

III. Serial Studies on a Consecutive Material of Partially Gastrectomized Subjects (Hofmeister Operation)

By

KAREN JOHANNE GRAVESEN[1]

In previous articles (17, 18) the author has described the method used in determining faecal fat content and in expressing the results, and presented the results from studies of 43 normal subjects. These studies covered a short observation period (10 samples from each of 10 healthy subjects) and further comprised a single sample from each of 33 other subjects. The conclusion drawn from the material was that total faecal fat content as percentage of dry weight in normal subjects increases with age and is adequately expressed by the equation

fat-percentage = 0.1214 × age in years + 5.63

In the present article the results from patients recovering from partial gastrectomy (Hofmeister operation) are presented. Complications in patients after partial gastrectomy may be negligible or on the other hand may be very serious. Among the most troublesome disturbances are the dumping syndrome and the weight loss often linked with reduced capacity for work. Lack of strength may also be due to anaemia which is not infrequent, and finally the stools may contain increased amounts of nitrogen and fat. In previous work concerning fat absorption after partial gastrectomy selected cases have been examined, usually because unpleasant or serious symptoms had arisen. Serial examinations of the same patient at various intervals after operation have not been carried out. In the present study a consecutive series of patients was examined, and serial studies were also made in each patient at various times after the operation.

PREVIOUS INVESTIGATIONS AFTER PARTIAL GASTRECTOMY IN MAN

Protein absorption tests and nitrogen balance studies in patients after gastrectomy will not be mentioned since no such studies were attempted in the present group of subjects.

Submitted for publication August 28, 1963.

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duodenal ulcer. The effect of the surgical procedure on the blood values was not expected until some time after operation — two-thirds of the patients were examined mor than tw years after operation. Postoperatively 89 men showed an average serum iron content of 103 µg/100 ml (µg %) (20—268 µg %) and 32 women showed 76 µg % (12—162 µg %). Ulcer patients on whom no surgery had been done and without haemorrhage had values of 124 µg % (61 men with range 42—356 µg %) and 112 µg (34 women with range 46—311 µg %). Smith and Mallett (34) gave labelled iron (ferrous sulphate perorally) to 19 patients before and 6 weeks as well as 6 months after Polya operation (6 cases) or Billroth I operation (13 cases) for ulcer. They admitted that the study had been done under ideal experimental conditions — perhaps the conclusions cannot be taken to bear on the absorption of iron from the food. They concluded that impaired ability to absorb iron seemed unlikely to account for the anaemia frequently found after partial gastrectomy.

Anaemia. Within one year after surgery 1 of 26 men and 1 of 9 women showed evidence of microcytic anaemia in Lyngar study. In the total series of 146 patients examined 1—6 years after the operation, 18 of the men and 37 of the women had hypochromic anaemia. I Rauch series of 893 patients the incidence of hypochromic anaemia was 17 with 2 cases of macrocytic anaemia reported. A review of the literature (7) indicated that about 15 of patients would show an iron-deficiency anaemia after the Polya resection. With regard to the frequency of pernicious anaemia, Lyngar found no cases of anaemia presenting signs of deficiency of the antipernicious factor. Ivy et al. (22) reviewed 1851 cases of partial gastrectomy for ulcer and found only 4 cases of pernicious anaemia (0..) various times after operation, which was less than the 0.3 observed in other patients showing gastric anacidity without gastric surgery. But according to Over-gaard Kristensen (39) Mollin nd Ross (?7) reported 22 patients, with megaloblastic anaemia unknown intervals following partial gastrectomy who had low values of vitamin B_{12} in serum. The average alue was 87 pg/ml, range 35—160 pg/ml. He reported 3 other patients who had low vitamin B_{12} alues 2—17 years after partial gastrectomy.

From the records of Medical University Department I at Aarhus Municipal Hospital for the years 1958 and 1959 the author found 10 cases in whom Polya operation had been done and who had their serum B_{12} values measured. Of these, 5 patients showed normal values (bove 200 pg/ml) 3—5 years after operation, 1 patient had a value of 170 pg/ml 9 years after operation, and 4 patients had abnormally low values 11, 16, 19, and 21 years after operation (124, 50, 50, and 112 pg/ml, respectively).

In 119 patients Lous and Schwartz (25) determined vitamin B_{12} absorption 1—35 years after partial gastrectomy and found 50 % of the patients showing low absorption, which, they wrote, may have been inaccurate.

2. *Previous fat absorption studies after partial gastrectomy*

Various methods have been used to investigate absorption of fats.

Vitamin-A absorption test. The immediate influence of gastric operations on serum vitamin-A levels was investigated by Popper et al. (29) who found no change in serum concentration of vitamin A. Although they made no actual vitamin-A absorption tests in patients after gastrectomy, they did vitamin-A bsorption curves in 10 ulcer patients treated medically and 6 patients with gastric carcinoma, all showing normal values. Adlersberg and Hammerschlag (1) made itamin-A absorption tests in 12 patients with tritional problems 1—21 years after partial gastrectomy for ulcer and reported results as normal for the ver age patient. Jones et al. (23) reported low values in 5 patients at intervals not stated after partial gastrectomy for ulcer. Serum vitamin-A values were improved by feeding an emulsifier (Tween 80) with the vitamin A. This finding suggests that hydrolysis may proceed more slowly in these patients than in healthy subjects. Finally Cournoulis and Neumayr (9) using itamin-A bsorption tests studied a group of 79 selected gastrointestinal patients, all without weight increase or even with loss of weight postoperatively. Results were expressed as "fat-absorption with an verage normal value of 96.15.

Twenty-one patients with Billroth II anastomoses howed an average value of 87.78 (mostly determined many ears after oper tion), but the values ranged widely ... the ...

1 Studies other than on fat absorption

The dumping syndrome By dumping syndrome should be understood early post prandial distress symptoms. Most frequently dumping distress occurs during the first two months after operation (5) Everson et al (12) reported a frequency of 50 8 in 222 patients 1—8 years after resection by a modification of the Billroth II operation. Waugh (37) verified that with the Hofmeister operation the proportion of permanent dumping syndromes was about one third of that seen with the Polya operation at the same clinic.

The Hofmeister operation is a modification of the Billroth II gastric resection in which after partial removal of the stomach, the cut end of the stomach is partly sewn together and the anastomosis with the jejunum is made to the remaining opening with exclusion of the duodenum. The proximal end of the duodenum is closed, but the duodenal contents can drain into the jejunum.

In the Polya operation the technique used is also that of the Billroth II resection, but the entire cut-end of the stomach is used for the anastomosis.

For detailed discussion of the problem of the dumping syndrome the reader is referred to original papers (2, 3 19 and 20 a. o.)

Weight loss Everson et al found that 78 8 of 222 patients weighed less than before operation. In reviews from the literature postoperative weight loss was recorded in 42 of 864 patients following partial gastrectomy for ulcer (22) Rauch (30) published an extensive study of 893 patients following a Hofmeister retrocolic operation for peptic ulcer with removal of three-quarters of the stomach and the use of a short efferent loop of intestine. He stated that 567 of 699 patients weighed less than before operation, while 132 gained or maintained weight. In 170 patients, 2—7 years after partial gastrectomy for ulcer largely Hofmeister resection Krieger Lassen (24) found the preoperative weight increased in 69 patients, and decreased in 51 In observations 1—7 years after Hofmeister procedures for ulcer two-thirds of 50 patients weighed less than their "ideal weight" (33) Some authors have believed that the failure to gain weight cannot be ascribed either to any abnormality in digestion or to abnormal absorption of fat and protein (4) but weight loss has been ascribed to loss of calories in the stools (11 38) although it was stated that such loss can be overcome if the patient ingests sufficient amounts of food. From studies in humans (11) it has been concluded that the caloric loss was sufficient to explain the weight variations found. Shingleton et al. (33) however emphasized that of 15 patients who maintained their ideal weight" after Hofmeister operation, 6 excreted large amounts of fat in their stools. Since no increase in metabolism has been shown, the conclusion reached has been that insufficient intake of calories leads to weight loss.

Intestinal transit time Rauch observed that patients with diarrhoea after gastric resection often had a rapid transit time as shown by gastrointestinal X-ray examination. After medication to decrease intestinal motility an increase in weight would frequently follow Thus changes in time for intestinal passage may play a rôle in complications arising after partial gastrectomy and anacidity has been suggested as a possible cause of the diarrhoea (32) Gordon Taylor et al (16) were the first to investigate the frequency of diarrhoea and constipation in gastrectomized patients and found that of 52 patients, 1—7 years after Polya operation for ulcer 6 had diarrhoea while 11 had constipation. In the literature, 15 patients with diarrhoea were reported from a total of 57 patients after Billroth II operation the cases of Gordon-Taylor et al excluded (38) The frequency of constipation was not recorded Krieger Lassen found 8 patients with diarrhoea and 18 with constipation among 163 patients after the Hofmeister operation, and Brunsgaard reported 8 cases with diarrhoea and 15 with constipation in a series of 184 patients treated for gastric or duodenal ulcer with the Billroth II operation. One of the patients was not considered cured because of the severe sustained constipat on The nature of the diarrhoea after partial gastrectomy has not been clearly defined Only Gavrila (15) described the appearance of "poudre de charbon 12 hours after ingestion as indicating a very accelerated food transit time. This acceleration was found in nearly all" of 13 patients after partial gastrectomy in 10 cases the faecal fat content was high, and in 6 cases extremely high (more than 20 g daily)

Serum iron content. Lyngar (26) studied various specific blood changes in 146 patients 1—6 years after partial resection for gastric or

odenal ulcer. The effect of the surgical procedure on the blood values was not expected until some time after operation — two-thirds of the patients were examined more than two years after operation. Postoperatively 89 men showed an average serum iron content of 103 μg/100 ml (μg) (20–268 μg) and 72 women showed 76 μg ** (12–162 μg %). Ulcer patients on whom no surgery had been done and without haemorrhage had values of 124 μg ** (61 men with range 42–358 μg **) and 112 μg (34 women with range 46–311 μg). Smith and Mallett (34) gave labelled iron (ferrous sulphate perorally) to 19 patients before and 6 weeks as well as 6 months after Polya operation (6 cases) or Billroth I operation (13 cases) for ulcer. They admitted that the study had been done under ideal experimental conditions — perhaps the conclusions cannot be taken to bear on the absorption of iron from the food. They concluded that impaired ability to absorb iron seemed unlikely to account for the anaemia frequently found after partial gastrectomy.

Anaemia. Within one year after surgery 1 of 28 men and 1 of 9 women showed evidence of macrocytic anaemia in Lyngar's study. In the total series of 146 patients examined 1–6 years after the operation, 18 of the men and 57 of the women had hypochromic anaemia. In Rauch series of 893 patients the incidence of hypochromic anaemia was 17 with 2 cases of macrocytic anaemia reported. A review of the literature (7) indicated that about 15 of patients would show an iron-deficiency anaemia after the Polya resection. With regard to the frequency of pernicious anaemia, Lyngar found no cases of anaemia presenting signs of deficiency of the antipernicious factor. Ivy et al. (22) reviewed 1851 cases of partial gastrectomy for ulcer and found only 4 cases of pernicious anaemia (0.2 various times after operation, which was less than the 0.5 observed in other patients showing gastric anacidity without gastric surgery. But according to Østergaard Kristensen (30), Mollin and Ross (27) reported 22 patients, with megaloblastic anaemia t unknown intervals following partial gastrectomy who had low values of vitamin B_{12} in serum. The average alue was 87 pg/ml, range 33–160 pg/ml. He reported 5 other patients who had low vitamin B_{12} values 2–17 years after partial gastrectomy.

From the records of Medical University Department I at Aarhus Municipal Hospital for the years 1958 and 1959 the author found 10 cases in whom Polya operation had been done and who had their serum B_{12} values measured. Of these, 5 patients showed normal values (bove 200 pg/ml) 3 5 years after operation, 1 patient had value of 170 pg/ml 9 years after operation, and 4 patients had abnormally low values 11 16, 19, and 21 years after operation (124 50, 50, and 112 pg/ml, respectively).

In 119 patients Lous nd Schwartz (25) determined vitamin B_{12} bsorption 1–35 years after partial gastrectomy and found 30 of the patients showing low bsorption, which, they wrote, may have been inaccurate.

2. Previous fat absorption studies after partial gastrectomy

Various methods have been used to investigate absorption of fats.

Vitamin-A absorption test. The immediat influence of gastric operations on serum vitamin-A levels was investigated by Popper et al. (29) who found no change in serum concentration of vitamin A. Although they made no actual vitamin-A absorption tests in patients after gastrectomy they did vitamin-A bsorption curves in 10 ulcer patients treated medically and 6 patients with gastric carcinoma, all showing normal values. Adlersberg and Hammerschlag (1) made itamin-A absorption tests in 12 patients with nutritional problems 1–21 years after partial gastrectomy for ulcer and reported results as normal "for the ver age patient". Jones et al. (23) reported low alues in 5 patients at intervals not stated after partial gastrectomy for ulcer. Serum vitamin-A values were improved by feeding an emulsifier (Tween 80) with the vitamin A. This finding suggests tha hydrolysis may proceed more slowly in these patients than in healthy subjects. Finally Courmoulis and Neumayr (9) using vitamin-A absorption tests studied group of 79 selected gastrointestinal patients, all without weight increase or even with loss of weight postoperatively. Results were expressed as "fat-absorption with an average normal value of 96.15

Twenty-one patients with Billroth II anastomoses showed an average value of 87.78 (mostly determined many years after operation) but the values ranged widely with one

normal one slightly subnormal and 2 extremely low tests.

Labelled fat absorption tests Following determination of blood radioactivity 4 5 and 6 hours after feeding labelled fats and in many cases determination of feacal radioactivity as well, Ruffin et al (31) reported that of 219 post-surgical ulcer patients 60 showed moderate or marked impairment of fat absorption following Billroth II procedures 46 patients were examined with I^{131}-labelled triolein as well as I^{131}-labelled oleic acid Although 30 subjects showed normal blood values after oleic acid only 16 had normal triolein values, perhaps due to an insufficient or slow hydrolysis. In 19 patients tabulated because of moderately or severely impaired fat absorption, faecal radioactivity varied from 3—62 of the dose given More than 2 excretion was considered abnormal No apparent relationship existed between blood values and faecal radioactivity Extremely low values of oleic acid were generally found in the stools. The same group reported 50 patients operated on with Hofmeister s technique showing radioactivity in blood following administration of labelled triolein with values slightly lower than the blood radioactivity levels found after Billroth I operations Correspondingly 8 patients after Billroth I operation had an average faecal content of 4 5 of the dose given, and 48 patients after Hofmeister resection showed averagely 14 0 in faeces. The range in each group was wide 0.2—8 7 and 0—57 4 (33)

Faecal fat excretion and fat balance studies 48 of 119 patients had neutral fat and fatty acid crystals in varying amounts in faeces (6) as estimated by microscopy Occasionally larger amounts of fatty acids and soaps have been reported (1) Rauch frequently found a decreased fat absorption in some patients unable to maintain or gain weight Gordon Taylor et al. (16) reported faecal fat content as percentage of dry weight in 25 of 52 patients studied. The method used was not stated, but probably 15 values should be considered abnormal and 3 regarded as borderline values as they were above the upper normal values found by Fowweather From intake-excretion studies in 12 patients at various intervals after partial resection for ulcer Gavrila and Damceo (15) found a faecal fat loss representing 7—46 % of fat ingested Faecal fat content ranged from 8—41 g daily and the lowest values were 8, 8 and 13 g Searching the literature Wollaeger et al. stated that the latter study was the only intake-excretion study in Billroth II-operated patients. Wollaeger s intake-excretion studies included 10 subjects in "good" condition and 4 clinically poor" cases after Polya operation. Generally speaking the fat loss was least in patients with mild or no digestive symptoms. The ten good" cases, who ingested 208 g of fat daily excreted 13.5—31.5 g of faecal fat (33 7—66 6 % o dry weight) Even with this large fat intake the lowest excret on was no higher than in the highest healthy control subject. With an intake of 100—125 g of fat the 3 "poor" cases excreted 11—20 g daily and the other poor" case 63 g (30 6—58.8 of dry weight) Daily average excretions from 7 to 19 g of fat have been reported in 4 patients after partial resection for duodenal ulcer the interval after operation not stated (23) In one subject the daily addition of 0 3—0.9 g of desoxycholic acid to the food had no effect on the amount of faecal fat. Another 4 emaciated patients excreted 6.2—14 6 g on an average daily These amounts were diminished by giving the Tween 80-emulsifier n doses of 1 5 g at meals 3 times a day One of the latter 4 patients showed a normal fat excretion, 3.2 g before operation. Probably as a result of low fibre in the ulcer diet given, the amount of dry substance in faeces was extremely small for which reason the percentage of fat was high, 26 One month after operation the patient excreted 10 8 g (26 of dry weight) and 7 months after operation 14 6 g (50 of dry weight) With Fowweather s method fat excretion has been found to be 11 3—22 8 of dry weight in 8 patients 6 months or more after various types of gastrectomy (4) These values are normal. Within one month after Polya operation normal fat excretion was demonstrated in 12 patients, 12 6 g ± 1 1 g in 3 days (7) but 11 other patients excreted abnormal amounts of fat (28 5 ± 10 2 g in 3 days) 8—11 months after the operation. In the same study 14 patients within one month, and 10 within 6—9 months after Billroth I operation, had normal faecal fat averages 6.1 and 8.8 g in 3 days, respectively

Thus many studies report an increased amount of faecal fat or other signs of disturbed fat absorption as estimated from blood stud

les, but no evidence is available as to how often such disturbances will develop in consecutive series of patients and how soon after partial gastrectomy faecal fat excretions may become normal — or abnormal. Nor has the possibility of relating the increase of faecal fat to an increase in the content of neutral fat been examined. This possibility should be of both theoretical and practical interest. It might also be of interest to follow the fat excretions of patients with persistence of dumping syndrome e. g. one year after operation, as well as to compare serum iron content with fat excretion. Long-term studies would, of course, be of the greatest interest, but in the following investigation patients were seen at regular intervals and up to one year after operation.

OWN INVESTIGATIONS

Material

Choice of patients. A consecutive series of 26 patients was studied following partial gastrectomy (Hofmeister operation) at the Central Hospital, Randers. The operations were performed from July 1st, 1958 to June 30th, 1959. Cancer patients in whom resection had been done in healthy tissue and who were judged free of metastases were included in the study. If no metastases were found in macroscopic examination of appropriate lymph nodes removed during surgery, if no liver metastases were apparent by inspection, and if laboratory values were normal for alkaline phosphatase and glutamic-oxalacetic transaminase in serum, the patient was considered to be free of liver metastases. Three patients were excluded after operation because of cancer: one had complicating cholangitis and diabetes, 2 patients died, and one patient refused any further examination 9 days after surgery. Pertinent details concerning the remaining 26 patients are presented below.

The subjects were examined 9 or 10 days, 6 weeks, 3 months, and approximately one year after operation. A vitamin-A absorption test was done at the first examination and repeated 3 months after surgery (detailed results of these tests will be published elsewhere). The remaining observations were undertaken after the patients had been discharged.

General conditions during the hospital stay

Before operation. Routinely a radiological examination was done, and whenever possible gastric acidity following an Ewald testmeal was determined. A Kemp test meal was given in order to measure gastric retention of food. A four, six or twenty-four hour X-ray examination following intake of a barium-suspension was also used for estimating the emptying time of the stomach. All necessary laboratory tests as well as the correction of low haemoglobin values with whole blood transfusions were carried out as soon as possible.

Operation. The operation used was a Hofmeister procedure, antecolic, i. e. the jejunum was brought up anterior to the colon. Care was taken to use a rather long afferent loop of intestine, fixing this at the lesser curvature of the stomach. As a measure of size the anastomosis would admit the end of a thumb.

Postoperative care. A stomach tube was left in place for approximately 80 hours after operation. On the day of surgery no liquid was given orally or by tube, and the stomach was emptied hourly, later at longer intervals. Water and light ale were given in increasing amounts from 15 to 40 ml once every hour orally for the next 3 days. Gradually tea was substituted for water and on the third day oat gruel and milk were given. After removal of the tube a liquid diet was taken. Generally about 4 days after operation a soft-boiled egg and white bread without the crust could be given for supper. The food was rapidly increased in amount and changed in type so that by the 9th postoperative day the patients were eating a light diet and the stools were passed normally. Postoperatively necessary laboratory tests were made and serum or blood transfusions were given. Usually the patients were discharged on the 10th day after operation. The patients were instructed to have their food carefully prepared, eat small and frequent meals, and avoid sugar and milk if not tolerated. This instruction of patients was not different from the usual ward routine. Each patient was asked to return for control 6 weeks after operation.

Sex, age, and duration of illness. Twenty-six patients were studied, 5 of whom were women. The average age at operation was 49 years, with the range from 23—70 years. The duration of the illness varied from less than a year

normal one slightly subnormal, and 2 extremely low tests.

Labelled fat absorption tests Following determination of blood radioactivity 4 5 and 6 hours after feeding labelled fats and in many cases determination of feacal radioactivity as well, Ruffin et al (31) reported that of 219 post-surgical ulcer patients 60 % showed moderate or marked impairment of fat absorption following Billroth II procedures 46 patients were examined with I[131] labelled triolein as well as I[131] labelled oleic acid Although 30 subjects showed normal blood values after oleic acid only 16 had normal triolein values, perhaps due to an insufficient or slow hydrolysis. In 19 patients tabulated because of moderately or severely impaired fat absorption, faecal radioactivity varied from 3−62 of the dose given More than 2 excretion was considered abnormal. No apparent relationship existed between blood values and faecal radioactivity Extremely low values of oleic acid were generally found in the stools. The same group reported 50 patients operated on with Hofmeister's technique showing radioactivity in blood following administration of labelled triolein with values slightly lower than the blood radioactivity levels found after Billroth I operations Correspondingly 8 patients after Billroth I operation had an average faecal content of 4 5 % of the dose given, and 48 patients after Hofmeister resection showed averagely 14 0 in faeces. The range in each group was wide 0.2−8.7 and 0−57 4 (33)

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Thus many studies report an increased amount of faecal fat or other signs of disturbed fat absorption as estimated from blood stud

Table II. Preoperative and operative details of patients studied

Case no. Sex	Age at onset	Age at oper.	Retention: X-ray (hrs)	Retention: Kemp test meal (6 hrs)	Indication for surg.	Part of stomach removed	Diagnosis at oper.
1 ○	27	44	—	None	—	2/3	Gastric ulcer
2 ○	22	23	—	—	Melaena	12 × 12 cm	Duodenal ulcer
3 ♂	48	50	Yes (24)	—	Stenosis	2/3	Duodenal ulcer
4 ♀	20–30	70	—	—	Cancer?	3/4	Carcinoma
5 ♂	59–60	65	—	None	Melaena, loss of weight	2/3	Chronic gastric ulcer
6 ○	34	44	—	None	Cancer	1/2	Chronic gastric ulcer
7 ♀	26	36	—	—	—	13 12 cm	Gastric ulcer (previous)
8 ○	34	44	—	None	—	11 13 cm	Chronic gastric ulcer
9 ♂	40	50	None (4)	—	—	13 12 cm	Gastric & duodenal ulcer
10 ○	26–27	37	None (4)	—	Melaena, previous	17 13 cm	Gastro-duodenitis
11 ♂	42	45	—	—	Haematemesis	12 16 cm	Gastric ulcer
12 ○	47	49	—	None	Pains	2/3	Gastric ulcer
13 ♀	39	59	Yes (6)	125 ml	Melaena	2/3	Chronic gastric ulcer
14 ○	15	43	—	None	Weight loss	1/2	Gastric ulcer
15 ○	42	55	—		Melaena	1/2	Pyloric gastritis
16 ○	24	28	—	None	—	3/4	Pyloric gastritis
17 ♀	55	63	—	None	—	1/2	Gastric ulcer
18 ♂	49–50	55	—	None	—	2/3	Gastric ulcer
19 ○	43	45	—	None	Melaena	2/3	Gastric ulcer
20 ○	68	69	—	—	Leiomyosarcoma/myoma	12 14 cm	Leiomyoma, -sarcoma
21	45	47		—	Haematemesis	14 14 cm	Gastric ulcer
22 ○	20	46		None	Melaena	2/3	Chronic pyloric ulcer
23 ♂	31	51	—	None	—	11 11 cm	Chronic duodenal ulcer
24 ○	22	30	—	40 ml	—	12 16 cm	Chronic juxtapyloric ulcer
25 ○	47	34	—	None	—	2/3	Pyloric gastritis
26 ♂	47	82	—		Melaena	1/2	Chronic juxtapyloric ulcer

Indicates that the pancreas was involved. Where no specified indication is mentioned, the long duration or recent aggravation of the condition led to operation.

Case 23. A severe condition with fever and retention developed immediately after operation with dilated duodenal stump. After 4 weeks the fever suddenly subsided and he quickly recovered.

Case 25. An operation for thyroid cancer had been performed 5 years before the stomach operation and partial removal of the right lung due to metastases had been made 3 years before the gastric resection. X-ray of the lungs at time of gastrectomy was satisfactory without signs of progression.

Case 26. A temperature rise for 18 days post-operatively was thought due to subphrenic abscess, which, however, could not be proved. The temperature fell, and he soon recovered.

The complications in the remaining cases were insignificant, and in most cases the post-operative period was completely uneventful. 23 patients left the hospital in 10—15 days after operation.

Table I Preoperative faecal fat determination

Case no.	Age (yrs)	Neutral fat (of dry weight)	Total fat (% of dry weight)
13	59	3.6	15.3
18	55	1.6	10.6
19	45	2.0	11 1
22	36	3.4	15.3
24	30	2.5	12.4

to 30 years. Ten patients, or nearly half the group, had symptoms for 10 years or more before operation, with an average duration of 11 years.

Faecal fat before operation Since the average hospital stay preoperatively was short, faecal anal ses could be done in only 5 cases. These were found completely normal (table I)

Retention and gastric secretion before operation. Gastric retention was infrequent. Among 20 cases examined it was demonstrated by X ray in only two cases, and in two other patients when the Kemp test meal was used with gastric lavage six hours after the test meal. Results are given in table II Fasting gastric secretion volume measured in the morning n 14 patients varied from 0 to 29 ml In 8 patients no free acid was found (negative reaction on Congo-paper) In 4 of these patients the gastric content was estimated following histamine injection in 2 cases giving negative reaction on Congo-paper Results of feed ng an Ewald test-meal to 15 patients will not be recorded in detail Only one patient had a definite and one patient a questionable hyper secretion (cases 10 and 9 respectively) Slight hyperacidity was found in cases 8 10 23 and 25 and no patient showed anacidity

Indication for operation Where no specified indication is mentioned (table II) the long duration or recent aggra ation of the condition led to operation Melaena or haematemesis were the sole o dominant indicat ons in 10 cases. In two patients cancer was suspected (one proved to be carcinoma) and on patient had a preoperative diagnosis of leiomyosar coma/leiomyoma, confirmed at operation. *Size of surgical specimen removed* This was either estimated by the operating surgeon (in 23 cases done by the same surgeon) or measured routinely at the pathological institute where all operative specimens were examined (table II)

Diagnosis Diagnosis (table II) was made by the operating surgeon or the pathologist or both. Where inflammation was considered of secondary importance, it has not been mentioned in table II Only 4 of the patients had duodenal ulcers (cases 2, 3 9 and 23) Three juxtapyloric or pyloric ul ers were found (cases 22, 24 and 26) The remaining 14 ulcers were all diagnosed as gastric ulcers.

Complications

Case 1 A severe hepatitis of unknown origin necessitated a prolonged stay in the medical ward, rehospitalisation starting 3 months after operation, and as the patient s general condition was very poor the examination at this time was omitted. It was decided to exclude him from further study However it later appeared that he had been in the medical ward again 11 months after operation, at which time his faecal fat content had been deter mined and his weight recorded. It was decided to include these figures in tables V and VI He had no hepatitis 11 months postoperatively

Case 3. Moderate and inconsistent fever for the immediate postoperative period with per foration of an abscess in the incision wound on the 13th day was followed by quick recovery

Case 4 Apart from a slight and superficial phlebitis in both arms postoperatively this woman stayed remarkably well with an excellent appetite and increasing weight until 7 months after operation She then quickly went downhill and died in hospital from her cancer 9 months after operation.

Case 10 Shortly after discharge the patient returned to spend a full month in the ward with pneumonia, for a time he also excreted pus w th the stools. After renewed discharge he had repeated gall bladder trouble without fever

Cases 13 and 14 These patients had complicating rheumatoid arthritis in case 13 t was progressive. An embolus was removed from the left iliac artery 5 weeks after operation case 14 at which time he lost considerable weight.

weight in normal subjects increases with age and is adequately expressed by the equation:

fat-percentage = 0.1214 × age in years + 5.63.

Results and discussion of studies other than those pertaining to fat absorption

One patient was not seen after discharge until one year after operation (case 26). In patient 3 the 6-week examination could not be performed. Case 4 died before the one-year examination. The exact number of patients at the various controls can be seen from table V. Except for those results pertaining to fat absorption which will be explained later, the individual results will not be given in detail. Since the prime interest is in faecal fat concentration, other laboratory results and the clinical observations during the whole period will briefly be presented together. Tables III and IV report some individual results in patients 3 months and 1 year after surgery.

Dumping syndrome. At 6 weeks the frequency of dumping syndrome was greater than at any other time. Eight of 23 patients reported one or several symptoms belonging to this syndrome once or several times during the last month (only patients 10 and 11 had moderate trouble). Most patients had slight symptoms exclusively after eating chocolate, sweet cakes or jam.

All 8 patients gradually improved, but 2 patients who were at first symptom-free later had slight trouble. The conditions of the patients at the one-year examination can be seen from table IV.

Six patients had dumping symptoms, one year after operation, more than once monthly. Only in case 10 could the symptoms be said to be of some importance. He had daily trouble. Case 15 deliberately avoided sweet food. Case 20 also avoided sweet cakes, although occasionally he might eat one without trouble. Eight patients showed no symptoms of dumping whatsoever.

"Small-stomach-trouble". Seven patients still had a feeling of epigastric fullness after meals, one year after operation. In all patients, except cases 10 and 12, this happened only when they ate "too much", particularly if they had a meal outside their home.

Intestinal passage. No patient complained of diarrhoea, and no one complained spontaneously of constipation. But when specifically asked if any further difficulties were present, cases 6 and 13 mentioned constipation. In case 13 this symptom was really troublesome. However, at the one-year examination, the constipation was completely controlled by means of linseed. As seen from table V, the transit time at that time was only 45 hours as compared with earlier findings of 110, 108 and 118 hours. In case 6 the constipation became gradually less pronounced, also by means of linseed.

Charcoal colouring indicated that 3 patients had a gastrointestinal passage time of less than 12 hours at any time after operation, 20 of less than 18 hours.

Weight. Each patient had his weight recorded by the 9th postoperative day. Although a few patients had gained some weight by the time of the 3-month examination (table III), 12 patients had gained less than 2 kg or had lost weight, probably due in some to complications (cases 10 and 14). Case 16 lost weight, probably because he changed to work with increased responsibility and strain. He worked as a farmer from the 12th day after operation.

At the one-year examination weight differences from the 9th postoperative day were recorded and results are given

Methods

1 Examination of patients

Clinical condition Rauch (30) cited several factors necessary in judging the clinical conditions of patients after resection. Ivy et al. (22) suggested classification of results. To ensure proper and uniform inquiry and recording a list was prepared (see below) and each patient was interviewed by the author with regard to each item on the list. Care was taken to distinguish between early and late postprandial symptoms.

Syndrome of the dumping stomach — early postprandial distress symptoms.

1 Weakness.
2 Dizziness.
3 Flushing
4 Sensation of warmth.
5 Perspiration
6 Palpitations.
7 Trouble after very cold food (ice) and very hot food (soup)

Syndrome of the small stomach.

8 Feeling of epigastric fullness
9 Nausea.

Miscellaneous

10. Rest after meals.
11 Lying down after meals.
12 Working capacity
13 Other symptoms complained of (late weakness)
14 Foods avoided, particularly milk and sugar

On the 9th day no use was made of this questionnaire

Definition of groups Classification of patients was made according to the following scheme

Group A. Symptom free or practically symptom-free
Group B. Small infrequent or negligible symptoms.
Group C. Condition better than before operation but not satisfactory
Group D No better perhaps worse, than before operation.

Intestinal passage As a rough indication of intestinal transit time 4 charcoal tablets were given with a meal and the interval noted until the faeces began to show charcoal.

Weight The patients were weighed without clothes in the ward, as long as they stayed in hospital but later weighing was done without shoes and overwear with a 1.5 kg deduction for clothes.

2 Laboratory methods

Routine pre and postoperative laboratory examinations were made without any participation by the author and are not recorded here

Haemoglobin value Using a colorometric determination, 100 being equal to 14.9? g Hb, duplicate determinations were routinely made and a deviation exceeding 5 was not allowed

Rauch considered a haemoglobin value below 12.4 g indicative of anaemia, which value corresponds to 83.1 Hb in the present study Arbitrarily then anaemia is considered to exist with values of Hb equal to 83 % and below

Serum iron. This determination was carried out only at the time of the one-year postoperative examination. Duplicate determinations including a frozen serum from a healthy subject and 2 different standards were always made. The method used was a modification of the method of Peterson (28) with normal values at the laboratory of 90—200 μg

Vitamin B_{12} in serum. This examination was likewise made only at the time of the one year postoperative examination. The lactobacillus leichmannii tube method was employed (21) normal values being 200—750 pg/ml.

Vitamin-A absorption test Determinations of vitamin A (correction for carotene included) were made with Sobel's method in the fasting subject and after feeding 200,000 I U vitamin A acetate in tablets with a fat-free breakfast, under standard conditions. No food or drink were allowed until the test was finished. Samples were taken 3 4 and 6 hours after the test meal Results in 50 healthy subjects had previously been obtained

Faecal fat determination. Normal values for faecal fat had earlier been established by the author using van de Kamer's method in examination of faeces from 41 subjects, eating any ordinary food without limit. A random sample of at least 50 g was examined in duplicate and results expressed as percentage of dry weight. The conclusion drawn from the material, consisting of 131 samples, was that total faecal fat content as percentage of dry

Table IV Patients one year after operation

Case no.	Increase from 9th day (kg)	Hb (%)	Serum iron (µg %)	B_{12} (pg/ml)	Working capacity	Symptoms more than once monthly	Group
2	0.9	78	79	208	Poor	5, 6	C (B)
3	7.0	109	276	504	Full	(8)	A
5	5.6	106	100	239	Full	(8)	B (A)
6	1.2	103	173	214	Full	—	A
7	4.8	83	83	251	Full	—	A
8	2.6	94	97	365	Full	—	A
9	5.0	93	160	231	Full	—	A
10	−4.6	84	179	276	Full	1 2, 5, 6, 8	B (C)
11	1.7	94	108	355	Full	1 2, 3, 4, 5, 6, (8)	B
12	4.5	98	219	576	Full	(8)	B
13	5.6	76	119	318	(50%)	—	A
14	3.5	107	104	185	(75%)	—	A
15	1.5	113	221	208	Full	None, but abstains from sweet food	A (B)
16	5.6	101	107	548	Full	8, occasionally	A (B)
17	6.0	83	127	298	Full	—	A
18	0.7	102	179	252	Full	2, 4 but weakness	B
19	7.7	72	151	152	Full	2, 3 4, 5	B
20	−0.4	98	102	239	(Full)	—	A
21	18.1	86	149	155	Full	—	A
22	0.5	78	83	182	Full	—	A
23	8.8	89	132	183	Full	—	A
24	0.7	105	163	395	Full	1 2, 4, 5, (6) 8	B
25	3.0	99	171	325	Full	8	B
26	17.0	109	176	378	Full	—	A

For list of symptoms and grouping see p. 428. Case 10 unduly tired after work. Parentheses indicate that the working capacity was as good as could be expected in view of intercurrent illness or age and habit. (8) indicates that the feeling of epigastric fullness as reported by the patient was very vague.

22 and 23). The five patients whose haemoglobin values indicated anaemic levels revealed one year after operation (cases 2 7 13 19 and 22) should be evaluated in relation to their serum iron levels.

Serum-iron level. This was determined only at the one-year examination. Five patients (cases 2 5 7 8 and 22) had abnormal serum-iron levels or fell in the low range of normal values (100 µg % or less). But only in cases 2, 7 and 22 was anaemia present. On the other hand in spite of satisfactory serum-iron content patient 13 was anaemic, probably due to her progressing rheumatoid arthritis. Patient 19 was also anaemic in spite of high serum-iron level.

Vitamin B_{12} value in serum. This determination was made only at the one-year examination. Since determinations were not made before operation it cannot be stated whether the low values found in

Table III Patients three months after operation

Case no.	Height (cm)	Weight (kg)		Hb (%)	Working capacity	Group
		9 days	3 months			
2	170	61.0	66.0	96	Fair	C (B)
3	184	54.0	57.5	111	Full	B
4	165	47.5	55.5	94	Full	A
5	172	59.0	62.5	95	Full, with care	B
6	165	70.0	75.0	105	Full	A
7	158	46.0	49.6	102	Full, with care	A
8	171	60.0	64.5	97	Full	A
9	171	60.2	65.5	106	Full	A
10	180	72.1	67.7	82	50%	B
11	179	59.0	60.4	93	50%	B
12	172	54.0	55.0	95	Full	A
13	159	41.2	41.0	86	(Scarcely full)	A
14	180	57.0	50.2	103	(50%)	B
15	170	79.0	79.5	95	Full	A
16	172	73.0	65.7	101	Full, with care	A
17	160	62.5	66.0	76	Full	A
18	169	51.5	52.5	105	Full	A
19	167	43.2	49.0	82	Full	A
20	163	57.0	57.0	102	(Full)	A
21	166	63.0	64.8	88	Full with care	A
22	163	57.0	57.0	77	Full	A
23	178	58.0	66.8	82	Full	A
24	182	65.0	67.5	110	Full	B (A)
25	168	65.5	67.0	107	Full, with care	B

For classification see p. 428. Parentheses indicate that the working capacity was as good as could be expected in view of intercurrent illness or age and habit. Case 2 was somewhat difficult, perhaps exaggerating symptoms, for which reason he may belong to group B, and in case 24 a better result than B was probable.

in table IV. Some had lost weight since the earlier examinations at 6 weeks and 3 months. In case 2 weight loss was probably due to external conditions with insufficient attention to food and meals. Patient 10 had real difficulty in getting a sufficient amount of food to put on weight in spite of numerous small meals daily. Case 14 had put on weight as judged from earlier examinations — his early weight loss had undoubtedly been due to the complications described. A weight increase as compared with the 9th-day level had not been attained.

Haemoglobin value. As stated anaemia is considered present with haemoglobin values of 83 and less. Haemoglobin results immediately following surgery are of no particular interest since transfusions were employed if indicated. Patient 21 had prescribed for him iron medication for some weeks after operation, but otherwise no medicine was given until at the 3-month examination when patients 17, 19 and 22 were advised to use ferrous tartrate tablets. Only patient 17 followed this advice. At the 3-month examination 5 patients were anaemic (cases 10, 17, 19,

Table I Total faecal fat in percentage of dry weight, intestinal transit time (hours) and final weight increase (kg)

Case no.	Age (yrs)	After op. 9 days Total fat	9 days Time (hrs)	6 weeks Total fat	6 weeks Time (hrs)	3 months Total fat	3 months Time (hrs)	1 year Total fat	1 year Time (hrs)	Increase (kg) after 9th day
1	44	34.5	—	—	—	—	—	18.6	—	3.5
2	23	43.3	—	16.7	—	12.7	95	17.8	43	0.9
3	50	34.1	—	(17.1)	—	18.1	—	13.4	—	7.0
4	70	30.8	—	28.7	—	26.1	—	—	—	—
5	63	29.6	24	26.0	—	20.5	46	25.0	16	5.6
6	44	16.1	64	23.0	66	36.7	42	17.6	49	1.2
7	56	16.4	20	12.5	12.5	13.9	18	6.9	13	4.8
8	44	20.6	72	7.2	44	13.8	19.5	15.4	58	2.6
9	50	28.1	34	20.3	43	23.8	44	18.2	43	5.0
10	37	19.8	10	14.6	21	21.6	23	21.6	18	−4.6
11	45	40.6	—	16.6	50	22.8	43	22.4	13	1.7
12	43	26.0	—	34.1	22	7.1	17	11.5	20	4.3
13	58	30.5	110	37.5	108	23.3	118	21.4	43	5.6
14	43	37.9	18	19.9	20	33.1	17.5	31.3	58	−3.5
15	53	15.2	58	24.4	46	20.0	42	8.7	33	1.5
16	28	68.4	—	22.0	—	12.0	50	19.2	43	5.6
17	63	23.9	14.5	12.2	—	32.4	36.5	18.1	9	6.0
18	55	25.8	18.5	14.7	23	24.1	20.5	12.0	20	0.7
19	45	32.6	—	38.4	—	20.5	72.5	31.8	41	7.7
20	69	44.8	22	16.6	14	28.1	12.5	25.1	14	−0.4
21	47	16.7	12.5	9.8	14.5	9.6	16	21.0	6	18.1
22	36	30.1	30.5	13.1	29	18.6	34.5	14.0	34	0.5
23	51	[1]60.9	22	26.1	—	26.5	—	26.6	22	8.8
24	30	36.2	17.5	18.1	19.5	17.1	57	22.4	14.5	0.7
25	34	24.6	17	14.5	37	15.4	18	19.6	15	3.0
26	62	—	—	—	—	—	—	15.3		17.0
No. of observ.		25		23		25		25		25
Means		33.3		20.3		21.3		19.0		3.7

With regard to missing chemical determinations see pp. 426 and 429.

Samples examined 20 days after operation, in case 3 not included in calculations.

Total fat 2.5 months after operation 18.3 % which is incorporated into the average, making 25 observations.

those tests had been normal, two-thirds of the observations could have been expected to fall within the range of the normal average ± the standard deviation, which had been previously determined in tests on 50 healthy subjects. Only one of the 19 tests gave a result above this range (case 8) 6 were within (cases 7 9 14 18, 20 and 22) but the remaining 12 (about two-thirds) fell below the range (cases 1

patients 19 and 21 are the normal levels for these patients or slightly decreased values None of the patients had extremely low values Possibly existing liver vitamin B_{12} deposits may have served to mask a decreased absorption of vitamin B_{12}.

Working capacity and subjective valuation Little difference was found one year after surgery compared with conditions 3 months postoperatively in capacity to do productive work. Although case 10 was content with the results of the operation the author considered his troubles severe enough to classify him in group C.

Generally speaking the frequency of the dumping syndrome one year after operation appears less than in the patients studied by Rauch (55 1—2 years after operation) and Everson et al. (50 8 %) The method of classification may be responsible since the presence of one of the symptoms listed as Nos 1—7 on page 428 was considered sufficient evidence of dumping in the studies cited At the one year examination in the present study 8 patients had no symptoms whatsoever of dumping And only in patient 10 and perhaps in case 11 could the dumping symptoms be said to be of any great consequence to the patient A comparison of occurrence and severity of the dumping symptoms one year after surgery with weight loss fails to explain the latter Of the 4 patients with weight loss at the one year examination (cases 10 14 16 and 20) only patient 10 had concomitant dumping symptoms The real cause for his weight loss however seemed to be his small stomach, which would accommodate only very small meals. Cases 2 6 11 15 18, 22 and 24 presented weight increases of less than 2 kg but only patients 2 18 and 24 showed some dumping and patient 11 had had somewhat more trouble Finally patient 19 while showing some dumping symptoms, increased his weight by 7 7 kg

Anaemia and low serum-iron values are likely to be present less frequently one year after operation than at longer intervals (26) The 5 patients who were anaemic correspond to less than the 42 per cent reported by Ivy et al One year after operation Lyngar found only one of 26 men and one of 9 women anaemic. The range of serum iron concentration is less wide than in Lyngar s cases, and the average in the author s cases is higher (144 μg /) It should be remembered that Lyngar s values for serum iron were obtained 1—6 years after operation.

No values for vitamin B_{12} have previously been established in patients one year after partial gastrectomy The values found in the present study are decidedly higher than those mentioned on page 423 in patients examined many years after partial gastrectomy

The finding of 92.6 % satisfactory results in 222 surviving patients (12) seems to indicate that the results in the present group are good Rauch s observation that "the typical patient would be able to do a full day s labor although with less endurance, would indicate that the number of patients able to do full work is satisfactory considering the degree of disability such patients had for years before the operation

Results of fat absorption studies

Vitamin-A absorption tests Details will be published elsewhere but results will be mentioned here to permit comparison with faecal fat concentrations.

Nineteen tests were completed 9 days after operation If the distribution of

Table VI. Dry weight of faeces and neutral fat in percentage of dry weight

Case no.	After op.							
	8 days		6 weeks		3 months		1 year	
	Dry weight of faeces (g/100 g)	Neutral fat (% of dry weight)	Dry weight (g)	Neutral fat (%)	Dry weight (g)	Neutral fat (%)	Dry weight (g)	Neutral fat (%)
1	19.2	1.3	—	—	—	—	29.9	3.1
2	20.0	4.1	43.2	0.9	33.0	2.3	34.9	3.7
3	21.8	3.6	20.7	(1.0)	28.4	2.8	16.6	2.7
4	22.0	3.4	32.7	1.5	33.0	2.6	—	—
5	24.1	2.9	21.1	3.4	34.3	1.2	30.2	1.2
6	17.7	2.2	38.9	1.1	38.9	2.5	30.9	2.1
7	16.0	2.4	31.6	2.0	24.7	3.8	24.0	1.8
8	24.2	2.1	40.4	0.9	25.7	5.4	27.2	1.9
9	29.5	1.9	29.1	0.7	36.4	3.2	25.9	2.3
10	20.9	3.2	26.2	1.7	23.4	2.3	22.9	3.1
11	21.3	4.0	31.4	1.4	56.1	2.3	30.0	2.5
12	17.4	1.6	28.7	2.5	33.1	0.0	25.7	3.6
13	23.8	1.5	39.7	2.7	53.3	2.5	44.4	1.9
14	14.8	2.1	27.5	2.5	30.1	2.9	25.9	2.9
15	23.6	2.6	31.1	2.8	31.1	1.9	32.4	0.0
16	22.3	8.2	28.2	3.4	32.0	1.4	33.5	5.9
17	31.5	2.5	58.0	1.8	31.5	5.6	29.8	2.2
18	28.0	2.3	23.8	2.2	30.3	2.4	28.4	0.7
19	24.8	3.0	33.4	3.1	33.9	1.6	29.8	2.7
20	30.2	2.5	23.0	1.9	21.3	2.1	18.7	7.2
21	23.1	2.3	28.6	1.1	26.3	2.4	31.6	4.1
22	25.6	3.0	34.5	0.5	36.5	1.9	33.9	3.6
23	43.4	3.1	21.3	1.7	21.9	2.7	20.5	5.4
24	19.9	(8.5)	23.4	0.9	31.3	2.2	24.6	4.1
25	31.6	2.2	21.4	2.2	31.6	2.0	16.8	7.0
26	—	—	—	—	—	—	14.9	1.9
No. of observ.		24		23		25		23
Range		1.3–8.2		0.7–3.4		0.0–5.6		0.0–7.2
Mean		2.8		1.8		2.5		2.9
NF (% of total fat)		8.5		8.9		11.7		15.3

With regard to missing chemical determinations see pp. 426 and 428.

Samples examined 20 days after operation in case 3 not included in calculations.

Neutral fat value 2.5 2.5 months after operation incorporated in calculations.

Value not acceptable, due to error in titration.

rarely to a value in the lower range of normal.

Neutral fat content at various times after operation. Even though the determination of neutral fat may be of occasional and limited value only it must be admitted to be of interest in this study. In table VI individual results for neutral fat in per

2 5 6 10 11 12 13 15 16 24 and 25) In 16 cases the tests were repeated 3 months after operation Figures are available for 10 of the 12 patients with low values in the first test, and 6 of these still showed values below the aforementioned range (cases 2 5 10 13 16 and 25) One result came within the range (case 6) and cases 11 15 and 24 had risen above this range. Five of the remaining repeats were all within the range (cases 7 9 18 20 and 22) with one above (case 19 whose first test was made a fortnight after operation)

It appears, therefore, that the conditions leading to a low vitamin A absorption have in some patients been abolished and a *higher result is found* in the second test. But it must be stressed that it is not possible to predict what will happen in the individual patient.

Faecal fat content In a few cases (see table V) determinations could not be made. In case 6 the 3-month examination which showed a value of 36 7 % was made just after New Year Another sample was examined before Christmas and had shown a faecal fat content of only 18 3 % Both figures are included in the statistical computation

Total fat 9 days after operation On the 9th day only patient 6 had a total fat content (recorded as percentage of dry weight) lower than at any other time after operation whereas 19 patients had highest percentage at this time The average *for the group* was high considerably higher than at any other time of study As later mentioned this might be due to an increased desquamation of cells because of the operation Therefore, an examination in the early postoperative period is not valid in estimating the later condition of the patient.

Total fat in later determinations However already 6 weeks after surgery many subjects have adjusted to their individual levels which they later maintained (cases 2 3 5 7 8 9 22 24 and 25) The number of patients showing moderate or high faecal fat is considerable From later examinations 3 months and 1 year after operation it appears that most patients maintain elevated values. Other patients in whom Hofmeister resections had been done years before (table VIII) also showed elevated values in the determination of faecal fat percentage.

It cannot be said that there is any definite improvement when the immediate postoperative examination is not included This is true not only for the group, *but also for the individual patient*, although values obtained at 6 weeks perhaps are less reliable for judging the future possibilities of the patients than later examinations.

Comparison of total fat values in patients with normal values When the total faecal fat percentages obtained in these patients are compared with the 131 results previously described the difference is evident, especially 9 days after operation. Although in patients 2 3 7 8, 22 and 25 faecal fat values have stabilized at the 6-week examination at levels below the highest normal values found (19 5 %) only the last four have normal values allowing for age. The faecal fat determination for patient 2 is clearly above normal limits and in case 3 the value falls between the 1 and 5 per cent limits Only a total of 6 of the 99 samples have values below the normal average (10 7 %)

No doubt can remain that in this unselected group of patients after Hofmeister operation most subjects have a considerably increased faecal fat content as compared with normal subjects, especially immediately after operation. In some patients the level returns to normal, but only

in the study of healthy subjects — 19.5 % total fat as percentage of dry weight.

These observations suggest that as indicated by both vitamin-A absorption test and faecal fat concentration there is in most cases an actual decrease in fat absorption immediately following partial gastrectomy (and perhaps other operations in the gastrointestinal tract). Furthermore, judging from 7 cases with better vitamin-A absorption tests there may be an increased amount of faecal fat due to causes other than decreased absorption.

It would be reasonable to suggest, as one possible cause an increased desquamation of cells from the intestinal tract, because of the operation, since cell-breakdown has been considered a factor contributing to faecal fat content (14).

Three months after operation in 13 cases the vitamin-A tests, whether high or low cannot be said to have any correlation with fat excretion. It would seem unreasonable to suggest a continued increase of desquamation at this stage as a cause of increased faecal fat.

Faecal fat content in relation to weight and dumping syndrome. There is no unanimity concerning the caloric loss which fat in the stools represents and whether it is related to weight loss. With the faecal fat levels in the present group no support can be found for a direct relationship between faecal fat content and weight loss, but the actual caloric loss cannot be estimated. Nor can any clear relationship be demonstrated with regard to the dumping syndrome and faecal fat levels (tables IV and V). Although patient 10 showed a weight loss and daily dumping symptoms, he had total fat percentage only just above normal range his main trouble was the small stomach, which necessitated the intake of very small meals approximately every two hours during the day. Patient 16 also lost weight while showing moderately increased faecal fat values at the later examinations. He had no dumping symptoms. Although patient 14 weighed less one year after operation than before, he had nevertheless increased his weight after the 3-month examination in spite of high faecal fat content. He had no dumping symptoms. With infrequent, but persistent, dumping symptoms patient 11 had also gained weight in spite of moderately increased faecal fat levels. The slight weight increase found after a year in patient 2 had no relation to faecal fat content which was normal. Actually he had lost weight after his initial recovery since he weighed 66.0 kg 3 months after operation but only 61.9 kg one year after operation. This was no doubt due to environmental factors. Finally patients 5, 9, 13, 19 and 23 gained weight in spite of more or less elevated faecal fat.

Faecal fat related to intestinal transit time and serum-iron. Gordon-Taylor et al. suggested an increased rate of flow through the intestines as the cause of increased faecal fat. This cannot be supported by the present study as no clinical diarrhoea was found and no relation is seen between transit time and faecal fat content. High faecal fat values are not necessarily found associated with quick intestinal passage (table V).

Serum-iron might be thought to be reduced to low normal or subnormal levels when faulty digestion and absorption exist. In the present study serum values below 100 μg% were found one year after operation. None of these values is, however accompanied by increased faecal fat.

Faecal fat values compared with those for other gastrointestinal cases (own figures). Tables VII and VIII present values from various gastrointestinal patients examined

centage of dry weight as well as for dry weight of faeces are given The latter will not be further discussed Note that the means of neutral faecal fat tend to be constant but the ranges are wide. Also in the individual patient the variation is considerable. If neutral fat is expressed as percentage of total fat, the amount of neutral fat is lowest immediately following operation and later it gradually increases, at the one year examination amounting to 15 3 % of total fat. The reason is that the content of fatty acids expressed as percentage of dry weight gradually decreases while neutral fat as percentage of dry weight remains fairly constant.

On 9th day 8 5 % NF/total fat
33 3 % total fat =
2 8 % NF and
30.5 % FFA.
At 1 year 15 3 % NF/total fat
19 0 % total fat =
2 9 % NF and 16 1 % FFA.
(NF = neutral fat FFA = free fatty acids)

It therefore seems most correct to express the neutral fat in percentage of dry weight as has been done in the present study when the total daily output of faeces is unknown By this way of expression it is shown more clearly that the main increase in faecal fat after Hofmeister operation is in fatty acids not originating from the determination of neutral fats in faeces but on the other hand that, on the average the higher neutral fat level after operation was maintained.

Comparison of neutral fat values in patients with normal values The averages for neutral faecal fat as percentage of dry weight in these patients as a group are at all times higher than in healthy subjects (1.27 % range 0 09—2.38) and the ranges are wider Also in most individual patients the variations at the 4 examinations are greater than in any of 7 healthy subjects with 10 examinations each. The first set of results showed a lower percentage for the ratio of neutral fat to total fat than found in healthy subjects — in 101 samples from 38 subjects examined by the author the average neutral fat was 11 9 % of total fat Gradually the normal value for the ratio is approached and the percentage even increases to 15.3 The reason for these alterations have been explained above.

Thus in comparison with normal values, fatty acids as well as neutral fat remain increased in patients after partial gastrectomy (Hofmeister operation)

Discussion

Relation of vitamin-A absorption to total faecal fat content as expressed in percentage of dry weight When the vitamin-A absorption test is related to faecal fat content, one expects to find high faecal fat values with low vitamin-A test results as an indication of poor absorption This is sometimes found Also low faecal fat levels occur with high results of the vitamin-A absorption test

In the first tests a fair correlation is found in 7 or perhaps even 10 of the 12 patients with low results (cases 1 2, 5 11 12 13 15 16 24 and 25) where the expected high faecal fat content is found. But in patients 6 and 10 a discrepancy exists where the fat content is low in relation to the vitamin A absorption tests. Furthermore, the remaining cases (8, 9 14 18, 19 20 and 22) with normal range or even high results in vitamin A absorption have at the same time more or less elevated faecal fat values. None of these values falls below the highest normal value found in the 133 samples examined

al. (7) in studies 1 month after Polya operation as well as 6—9 months after Billroth I operation. They correspond with values found by Butler et al. 8—11 months after Polya operation.

Regarding possible causes for the results found. The present study was not planned to explain the causes of a decrease in fat absorption after partial gastrectomy rapid emptying of the stomach, intestinal hurry diminished stimulation of pancreatic and bile secretions, inadequate mixing of digestive juices with the food (7 9 12, 38 and others) and "blind loop syndrome" due to the duodenal stump However Butler believed that the use of a long afferent loop was likely to cause an increase of faecal fat. The long afferent loop used in the operative technique of the present study may imply a longer surface for the digestive juices to traverse before mixing with food. Bacterial flora might be of importance by increasing faecal fat content as suggested by Frazer et al. (14) following intubation studies in achylic subjects, and by Cameron et al. (8) studying "blind loops" in experimental rats. Perhaps secretion processes are different from those in healthy subjects and may add fat not reabsorbed (14 and 36)

The only observations made in this study which might be pertinent to the causes of decreased absorption after the Hofmeister operation are the discrepancy evident t the 3-month examination between the vitamin-A absorption tests and faecal fat contents, and possibly the relationship of neutral fat to total fat.

I some cases high faecal fat content was found with high values for the vitamin-A absorption test If the absorption of vitamin A as employed in the tests i. e. as an ester of a short-chain fatty acid) can be considered to depend on the same digestive and absorptive processes as food fat, the discrepancy may imply that the increase of faecal fat is not entirely related to defective fat absorption. This discrepancy cannot be adequately explained at present. Perhaps the discrepancy is a consequence of the use of a vitamin A ester with a short-chain length. There is a possibility that the use of esters with the same chain length as those most commonly found in food fats might allow predictions on fat absorption to be made from vitamin-A tests. Also the use of esters as compared with an alcohol of vitamin A might help answer the question regarding hydrolysis. A slower hydrolysis was suggested to be responsible for lower values of vitamin A in blood following the feeding of vitamin A. A change in digestive conditions might also be the cause of the less frequent occurrence of normal triolein blood tests compared with the oleic acid tests used n patients after partial gastrectomy (33) If delayed hydrolysis were a possible cause of the results found, some hydrolysis might take place so late that much of the fatty acids could not be absorbed and moreover a larger amount of neutral fat than usual would pass unsplit.

In this Hofmeister group the neutral faecal fat levels show a small but definite increase in the excretion of unhydrolyzed fat in most patients at all intervals examined after operation. On the whole this increase is of the same magnitude in observations at various times after operation as expressed in percentage of dry weight. Variations in the individual as well as between individuals are, however great. The acceptance of a delayed hydrolysis seems reasonable, as the rapid emptying of the tomach does prevent the proper and gradual mixing of food and digestive juices — in spite of the slowing effect on emptying of the relatively small opening

Table VII Total faecal fat percentage Unoperated ulcer cases

Case no.	Sex	Fat (% of dry weight)	
		Gastric ulcer	Duodenal ulcer
1959			
518	♂	—	21.0
589	♂	17 4	—
1 152	♂	—	8.8
1,553	♂	—	10.8
2,806	♂	—	15.0
3,048	♀	—	11.5
3,082	♂	—	22.6
4,994	♂	—	19.4
13	♀	—	15.3
18	♂	10.6	—
19	♂	11 1	—
22	♂	—	15.3
24	♂	—	12.4

Cf. table I

Table VIII Total faecal fat percentage. After operation (usually for ulcer)

Case no.	Sex	Fat (% of dry weight)	
		Hofmeister	Polya
1957			
1 607	♂	22.5 (2)	—
—	—	31 4 (2)	—
1,919	♂	31.6 (2)	—
1958			
5,973	♀	11.9 (1) dumping	—
7 738	♀	—	23.5 (7)
1959			
278	♂	19.2 (/₂) dumping	—
994	♂	22.1 (8¹/₂)	—
1 129	♂	—	22.4 (10)
1,5.3	♂	—	20.0 (6)
1,875	♂	—	17.2 (7)
2,591	♂	30.1 (6)	—
7 113	♂	23.9 (1 /₂)	—

Dyspeptic symptoms.

Only slight or quit recent.

Mean of 5 determinations on consecutive days (15.1 22.0 32.1 23.3 and 26.8 %)

Parentheses indicat years after operation.

at the Central Laboratory Central Hospital Randers. Postoperative observations are recorded for patients other than the particular Hofmeister group studied. Unoperated ulcer patients usually have a faecal fat content within normal range during hospital investigations (table VII)

The values presented in table VIII are on the same level as in the particular Hofmeister group of 26 patients, and the tendency to maintain elevated faecal fat levels after Hofmeister operation evidently persists for years. This observation was made also after the Polya operation

Faecal fat values compared with earlier studies Ivy et al commented that in most cases the elevation of faecal fat excretion in patients after partial gastrectomy was small. As patients earlier studied mainly were selected cases examined because of some complaint or complications, one would not expect to find abnormal faecal fat levels in so many patients in a consecutive series as this group of 26 Hofmeister patients. It, therefore seems surprising that most of the patients have increased faecal fat values. In the consecutive series of 52 patients studied by Gordon-Taylor et al quantitative fat studies were made in 25 patients, 15—18 of whom showed abnormal fat excretion. These results seem to be consistent with the author's figures. The faecal fat values found in the Hofmeister patients presented here are higher than those reported by Babb et al. (4) from 8 patients after various gastrointestinal operations. Also the values are higher than those reported by Butler et

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of the stoma used in these Hofmeister patients.

Thus the final problem may be proper digestion rather than proper absorption. Consequently further serial studies would be of interest to elucidate this question using emulsifiers given to gastrectomized subjects with the meals. No doubt the chemical determination of faecal fat may still contribute to the understanding of the effect of gastric resection on faecal fat content along with other ways of studying fat absorption

Summary

Twenty five patients have been followed for one year after Hofmeister operation with special attention to the question of fat absorption

Clinically the frequency of dumping syndrome was low and with one exception the working capacity of the patients was satisfactory one year after operation. At that time 5 patients were considered anaemic. Three anaemic patients had a normal serum-iron content, and two other patients without anaemia had serum iron in the lowest range of normal values.

Two patients had rather low levels of vitamin B_1 in serum, 152 and 155 pg/ml — one of these had anaemia. Both patients had a high level of serum iron

Fat absorption as judged by the vitamin A absorption test was impaired in many patients 9 days as well as 3 months after the operation but no clear relationship could be shown with the faecal fat content.

Total faecal fat expressed as percentage of dry weight, and neutral fat in faeces remain increased in patients after Hofmeister operation. Only in a few cases did the total faecal fat return to a normal level, and extremely few values returned to a level below the normal mean.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Quantitative Determination of Plasma Phospholipids in Pregnant and Non pregnant Women, with Special Reference to Lysolecithin

By

Olle Vikrot

In most studies on blood lipids only the total amount of phospholipids has been measured. During recent years chromatographic methods have become available which permit separation of the phospholipids in plasma but so far only few quantitative studies have been published.

Observations on the amount of lysolecithin in human plasma range from no lysolecithin reported by Hanahan et al. (13) to 6—18 per cent of total phospholipids reported by several other authors (3 6, 9 21 22, 25 28 31 38).

Lysolecithin has a strong surface activity in water solutions (32) and could therefore be expected to play an important role for the physical state of the lipoproteins and blood cells. Small variations in the lysolecithin concentration can be expected to change this state. A more detailed knowledge of the normal lysolecithin level in blood and the concentration changes during different pathological conditions therefore is of great importance.

Submitted for publication September 6, 1963.

The aim of the present investigation was to find a method for the determination of lysolecithin and the other plasma phospholipids in amounts of blood small enough to allow repeated determinations in the same patients. The method was used in a study of the concentration of lysolecithin and the other main plasma phospholipids in pregnant women and in non-pregnant healthy controls.

The results presented below show distinct changes in the phospholipid composition during pregnancy especially a decrease of lysolecithin.

Material and methods

All chemicals used were reagent grade. The organic solvents were used without further distillation. The silicic acid (Bio-Rad Laboratories, Richmond, California) used for column chromatography was activated at 120° C over night.

Lecithin and phosphatidylethanolamine were prepared from egg yolk by the method of Lea et al. (16 30) lysolecithin and lyso-

hours in order to get better separation of the phospholipids.

The phospholipid spots were made visible by exposing the plates to iodine vapour (19). The spots with the main phospholipids and the corresponding blank areas were scraped off into glasstubes for phosphorus determination.

Phosphorus determination

Phosphorus was determined without prior elution of the phospholipids. The procedure was mainly according to Bartlett (1). However the digestion was performed with 0.2 ml concentrated sulphuric acid in sand bath at 220–240° C for 45 min. (24). After the colour development the tubes containing silica gel were centrifuged for 5 min. at 3,600 × g in an angle centrifuge. The supernatant could then be decanted into cuvettes and the absorbancy read in a Beckman B spectrophotometer at 830 mμ. The lecithin spots were so large and contained so much phosphorus that for their determination double amounts of all reagents were used. At each determination duplicate reagent blanks and standards (KH_2PO_4) were also run.

Calculations

The molar percentage of the different phospholipids within the phospholipid fraction was calculated by adding the phosphorus in the four main spots and determining the percentage each phospholipid contributed to this sum. The absolute values per ml plasma were calculated from these percentage values and the analyses of the total lipid-soluble phosphorus in the original plasma lipid extract. The 10-μl samples were used to determine the recovery of the applied phosphorus. The percentage values reported here are the mean of four determinations (duplicate separations on each of two extractions). Statistical treatment was carried out according to Hyrenius (15).

Comments on the method

Blood sampling

Plasma and serum may not have the same phospholipid composition. During coagulation phospholipids are liberated from the blood cells, especially from the platelets, and phospholipids may be bound to the clot. Furthermore, during the coagulation period rapid cooling of the blood is not possible because of haemolysis. Enzymic activity can be expected to go on for some time and change the phospholipid composition in serum compared to plasma.

The data in the literature does not give conclusive information. Gjone et al. (9) found no difference between serum and heparinized plasma. Marinetti et al. (21) found "qualitative changes" when heparinized instead of oxalated plasma was used but no details were given. Vogel et al. (38) compared serum and plasma obtained with several different anticoagulants and found no substantial differences. However among 14 determinations on the same blood heparinized plasma extracted 15 min. after drawing the blood showed the lowest lysolecithin value (the other extractions were made after 4 hours).

In the present study serum and plasma were compared in 12 cases. Serum was obtained by allowing the blood to coagulate for 2 hours at room temperature (18–20° C). Plasma was obtained by collecting 9 volumes of blood in 1 volume of ice-cold 0.1 M sodium citrate solution, using siliconized needles and glass-tubes in order to minimize

Table I. The plasma phospholipid composition after incubation of blood at room temperature (23° C) for various times. Each value is the mean of four determinations. Total lipid phosphorus was 103.2 μg/ml plasma

Time of incub. (min)	% of total phospholipids			
	PE	Lec	Sph	LL
0	3.3	68.9	21.6	6.1
10	3.5	68.5	21.5	6.5
30	3.3	68.9	21.3	6.5
60	3.7	68.8	21.2	6.4
120	3.4	68.3	21.6	6.7

PE = phosphatidylethanolamine
Lec = lecithin
Sph = sphingomyelin
LL = lysolecithin

phosphatidylethanolamine by the action of Crotalus adamanteus venom (Ross Allen's Reptile Institute Silver Springs, Florida) on the diacyl compounds according to Rouser et al (33) Sphingomyelin was prepared from bovine erythrocyte lipids through partial purification on a silicic acid column (13) From this column the neutral lipids and the ninhydrin positive phospholipids were eluted with chloroform methanol 4 1 (v/v) Further elution with methanol gave a fraction which on thin layer chromatography gave a faint spot with the mobility of lecithin and a very large spot which gave a positive choline reaction. After mild alkaline hydrolysis of this fraction with 0.5 N NaOH in methanol (10 min 27 C) (23) the large spot remained unchanged and the alkali-stable fraction was taken to represent sphingomyelin.

Blood sampling

About 6 ml of blood was taken from an antecubital vein into tubes containing 100 IU dry heparin (AB Vitrum Stockholm) immediately cooled in ice water or in a refrigerator and then centrifuged at 4 C and 1 900 × g for 10 minutes.

Extraction of lipids

Duplicate 1 ml samples of plasma were added drop by drop with shaking to 5 ml methanol in a 50 ml Erlenmeyer flask. After 10 min. at room temperature 5 ml chloroform were added and the contents mixed After another 10 min the mixture was filtered through filter paper (Munktell 00 previously washed in chloroform-methanol 2 1 (v/v)) into a 25 ml glass-stoppered measuring cylinder The flask and the filter were washed first with 5 ml chloroform and then with 5 ml chloroform methanol 2 1 (v/v) The filtrate was made up to 20 ml with chloroform methanol 2 1 (v/v) and 5 ml of 0 001 M CaCl solution was added The cylinder was vigorously shaken for about 15 sec. and then left over night in the dark at 4 C. The upper phase was removed by suction and the walls of the cylinder and the interface were washed twice with about 3 ml blank upper phase (methanol-0 001 M $CaCl_2$-chloroform 42 56 2 (v/v)) The lower phase was made up to 25 ml with chloroform methanol 2 1 (v/v) From this extract duplicate 1 ml samples were taken for determination of total lipid-soluble phosphorus. The extract was then stored in the dark at 4 C.

Thin-layer chromatography

The chromatography was performed on ordinary glass plates measuring 13×20×0 4 cm. They were coated with Silica Gel H (E. Merck AG Darmstadt) according to Lees & De Muria (17) Double layers of surgical adhesive tape were fastened at each side. The slurry was prepared by mixing 6 g silica gel with 15 ml water in an Erlenmeyer flask and shaking vigorously for at least 1 min. The slurry was spread on the glass-plates by a glass rod. After airdrying at room temperature over night the plates were heated at 120° C for 1 hour and then stored over silica gel in a glass jar

An aliquot of the plasma extract containing 40—50 μg of phosphorus was prepared for chromatography by taking it to dryness on a rotary evaporator under reduced pressure on a 40 C water bath. The removal of the last traces of water was aided by adding 5 ml of chloroform-methanol 2 1 (v/v) The residue was dissolved in 2 ml of chloroform and pipetted into a small stoppered glass tube The bottle was washed twice with 1 ml of chloroform The chloroform extract was evaporated in a stream of nitrogen at a maximum outside temperature of 40° C and immediately redissolved in about 0 12 ml of chloroform. From this solution 100 μl were aspirated into a microsyringe (Hamilton Company Inc. Whittier California) Two 10-μl aliquots were taken for phosphorus determinations. Two 40-μl aliquots were used for chromatography each on a different plate The aliquots were applied as 1 1/4 cm wide bands 1 5 cm from the lower edge of the plate A stream of cool air aided the evaporation of the solvent On each plate four aliquots could be separated while two lanes were run as blanks.

The chromatography was performed in glass chambers 7×15×20 cm at 4 C in the dark. The solvent used was 52 ml of chloroform methanol-water 75:25 4 (v/v) (7) The solvent had reached the upper edge of the plate after 2—3 hours but the chromatography was allowed to continue for 6—7

…st years various chromatographic methods …ave been used, especially silicic acid …olumn chromatography (9 24 25 28) …hich however does not adequately separat… …ecithin from sphingomyelin and sphingo…myelin from lysolecithin. Paper chromatog…raphy especially on silicic acid impregnated paper has also been widely used (20, 22, 38) The capacity of the paper does not allow th… application of sufficient amounts of phospholipids to allow accurate determination of phosphatidylethanolamine by this method.

Separation of phospholipids by thin-layer chromatography was introduced by Wagner et al. (39) The preparation of thin-layer plates is easy The plates allow separation of rather large lipid samples, thus permitting the determination of phosphatidylethanolamine with reasonable accuracy In comparison with column chromatography thin-layer chromatography is much more rapid and the separation between the various lipids is much more distinct.

The simple method used here of preparing thin layers was found to give as good results as the use of commercial …pplicators. The layers were about 0.3 mm thick in the dry state. Thinner layers had too low capacity while thicker layers tended to give poor separations.

Silica Gel H (which does not contain calcium sulphate) was used instead of Silica Gel G as it seemed to have … greater separating capacity and gave more sharply limited spots (19) Skipski et al. (35) have also found non-calcium-containing silica gel advantageous for chromatography of phospholipids.

The …pplication of the extract in the form of … band allowed separation of greater amounts of phospholipids than application in spots. Rather than to dissolve the lipids before chromatography in … very small …olume of … less volatile solvent, it was …hosen to use … larger volume which is easier to measure accurately Chloroform was used rather than chloroform-methanol mixtures as these tended to spread the phospholipids already at the application of the sample thus giving larger spots. The use of chloroform necessitated however the …ransfer of the extract to … glass-tube and the use of … microsyringe which could hold the whole volume to be applied, as the evap-

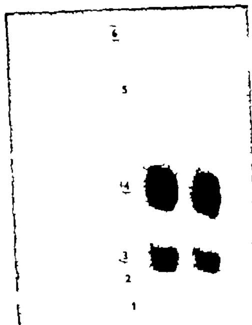


Fig. 1 Thin-layer chromatography of plasma phospholipids. Left: pregnant woman right healthy control. The amount of applied phosphorus was 15 µg in both cases. 1 possibly phosphatidylinositol. 2 lysolecithin. 3 sphingomyelin. 4 lecithin. 5 phosphatidylethanolamine. 6 probably free fatty acids. The spots were made visible by sulphuric acid spray and heating (19)

oration of solvent between repeated fillings of micro-pipettes introduced inaccuracies.

The chromatography was performed in the dark and at low temperature to prevent possibl… breakdown of phospholipids.

For qualitative runs there was a good separation of the main phospholipids within …bout an hour However for the separation of greater amounts of plasma phosphol pids in order to get accurate values for lysolecithin and phosphatidylethanolamine it was necessary to prolong the chromatographic run. It was found that the separation of the phospholipids continued after the solvent front had reached the upper edge of th… plate. Lining of the chamber with filter paper was found unnecessary in fact lining gave not so good separations as the phospholipids travelled much slower

the possibility of in vitro changes (37) The amount of lysolecithin was as a mean 10 % higher in serum.

In another experiment blood from a healthy individual was withdrawn into 5 heparinized tubes. The blood in one tube was immediately cooled centrifuged in the cold and extracted. The other tubes were left at room temperature (23 C) for varying times before centrifugation and extraction. The results are given in table I It is seen that the increase in lysolecithin after 2 hours is about 10 % of the original value

In 10 cases citrated plasma (obtained as above) was compared to heparinized plasma and the amount of lysolecithin determined. The mean difference was less than 1 %. Heparinized plasma was preferred in this study as the preparation of the sample is easier On microscopical examination only a few platelets were seen in the plasma obtained under these conditions.

It is known that when blood is incubated at 37 C there is a rapid rise in the lysolecithin concentration with a concomitant fall in the lecithin concentration (6 10) It appears that this process is going on also at room temperature. It thus seems necessary to prepare the lipid extract as soon as possible after withdrawal of the blood.

Extraction of lipids

The method of extraction is a modification of that of Sperry and Brand (36) The completeness of the extraction was tested by pooling the protein residues from 10 extractions in 50 ml chloroform methanol 1 2 (v/v) and boiling under reflux for 2 hours. After filtration the filtrate was evaporated to dryness and reextracted with 10 ml chloroform. The chloroform solution was filtered through glass-wool Aliquots were taken for phosphorus determination and the rest of the extract evaporated in a stream of nitrogen to a small volume and then analysed by thin-layer chromatography The plasma protein residue was found to yield on re extraction less than 0 1 of the phosphorus in the original plasma lipid extract With thin layer chromatography faint spots were observed on the place of the main phospholipid spots. On phosphorus analysis this was mainly sphingomyelin and lecithin while the lysolecithin and phosphatidylethanolamine spots gave values only slightly exceeding the blank values.

Washing of the lipid extract

It was found necessary to purify the extract according to the method of Folch et al. (8) If the initial extract was taken to dryness without washing there was often a distinct increase in lysolecithin with a concomitant decrease in lecithin. The unwashed extract thus seems to include some substance that makes the lecithin prone to breakdown. The washing removed 1—2 of the total phosphorus.

As lysolecithin is water-soluble the upper phase was investigated for the presence of phospholipids. Pooled upper phases (including the washing fluid) were divided into two equal parts and to the one part was added a small amount of lysolecithin. Both parts were then shaken with 1/5 volume of chloroform in a separatory funnel. The chloroform phase was collected and the washing with chloroform was repeated twice The pooled chloroform phases were then evaporated and analysed for phospholipids by thin-layer chromatography The recovery of the added lysolecithin was about 90 %. It was found that no more than about 4 % of the lysolecithin in the extracts was recovered in this way from the upper phase and still smaller amounts of the other phospholipids.

In another experiment lysolecithin corresponding to about 1/3 of the amount in 1 ml plasma was added to duplicate extraction mixtures after plasma had been added to methanol. Duplicate samples of the same plasma were extracted in the ordinary way It was found that the mean recovery of the added lysolecithin was 119 %

Thin-layer chromatography

For the fractionation of plasma phospholipids many methods have been used. Earlier methods depended on different solubilities of various phospholipids, estimation of the nitrogenous components etc. but are now known to give great methodological errors. Partial hydrolysis with estimation of the hydrolysis products has also been widely in use (3) but these methods can not separate between lecithin and lysolecithin. During the

In addition to these main spots a faint spot was regularly observed between the start and the lysolecithin spot. It ga a positive reaction with ammoniacal silver nitrate solution (34) and it possibly represents phosphatidylinositol. This component was also eluted from silicic acid and aluminium oxide columns in about the same way as phosphatidylinositol (12). Its phosphorus content was not routinely determined as t was found to gi an absorbancy only slightly exceeding that of the corresponding blank area. It thus accounts for less than 1 % of the applied phosphorus. Sometimes a faint iodine colour was given by the start area but not more than 1–2 % of the applied phosphorus remained at the origin. It probably represents water-soluble phosphorus-containing contaminants which have not been fully re moved by the washing procedure.

In order to see whether the phospholipid spots could be further separated two-dimensional chromatography was performed on 20×20 cm plates. The first run was per formed with the ordinary solvent. After short air-drying second chromatography was run with chloroform-methanol-water-concentrated ammonia 75 25 3 1 (/v) (34). Only one new and ery faint spot was observed. It had separated from the lecithin spot and probably represents lysophosphatidylethanolamine as it was ninhydrin positive.

Phosphorus determination

Thin-layer chromatography of phospholipids has mainly been used for qualitative purposes and only few quantitative investigations on blood phospholipids have been made (5 11 31).

A direct determination of phosphorus, without prior elution of the phospholipids was first used by Habermann et al. (11) and was found to be convenient. Elution of the spots with methanol, or methanol-chloroform and phosphorus analyses on the eluate gave much less recovery at least in the case of lysolecithin.

I order to investigate the recovery of phosphorus by this method unspotted plates ere run in the usual solvent system and for the usual time. After drying, areas of the silica layer of different sizes (1 4 resp. 8 cm²) were scraped directly into tubes and different amounts of lecithin solution (con-

Table III. Standard error for a single determination of phospholipid percentage

	PE	Lec	Sph	LL	No. of 4 determin.
Pregnant	0.14	0.92	0.98	0.30	5
Non-pregnant	0.28	0.60	0.64	0.31	9

Symbols as in table 1.

taining 0.5–12 μg of phosphorus) were added. After evaporation of the solvent, phosphorus determination was performed in the usual w y. Blank areas were also run. It was found that the mean recovery was 94.9 % (range 86.8–105 0 n = 32). There was no consistent ariation with small or large amounts of either silica gel or lecithin. With large amounts of silica gel or lecithin t was necessary to perform the phosphorus determination with double amounts of all reagents in order to get a complet digestion and bsorbancy in the linear part of the standard curve. With ordinary amounts of reagents the bsorbancy was linear to at least 6 μg of phosphorus and with double amounts of reagents thus to 12 μg. Th blank value of 1 cm² silica gel corresponds t less than 0.1 μg phosphorus.

The recovery of the phosphorus by chromatography was 91 6 ± 3.7 (S D) % (n = 56). The loss can be explained partly by the fact that some phosphorus was retained at the origin, and that the minor phospholipid below lysolecithin was not determined partly by losses while scraping off the spots. How ever the recovery compares well with that obtained by other methods of phospholipid separation, e. g. silicic acid columns and chromatography on silicic acid impregnated paper.

Very recently Dobzaki and Zieve (5) have published method similar to that described here. Silicic cid interfered however in their phosphorus determination and corrections had to be mad for different amounts of silica gel.

Robinson nd Phillips (31) have also described method for the determination of serum phospholipids by thin-layer chromatog raphy. These uthors after scraping off the

Table II The plasma phospholipid composition in 5 pregnant and 9 non-pregnant women. Each value is the mean of 4 determinations

Subject	Age	Total lipid P (µg/ml)	% of total P-lipids				µg P/ml			
			PE	Lec	Sph	LL	PE	Lec	Sph	LL
Pregnant										
K. B. O	18	132.3	4.5	72.2	22.4	0.9	5.9	95.5	29.7	1.1
M J	30	138.3	4.8	71.3	22.3	1.5	6.7	98.7	30.9	2.1
M. R.	20	126.4	4.7	74.5	19.2	1.7	6.0	94.2	24.2	2.1
D J	37	141.8	5.5	71.0	22.3	1.2	7.8	100.7	31.6	1.7
M. L.	29	117.4	4.3	74.0	20.4	1.4	5.0	86.8	24.0	1.6
Mean	27	131.2	4.8	72.6	21.3	1.3	6.3	95.2	28.1	1.7
Non-pregnant										
G L.	23	100.1	2.4	68.2	23.8	5.7	2.4	68.3	23.8	5.7
A. C. K.	24	76.0	4.0	68.5	22.6	5.0	3.0	52.1	17.2	3.8
L. K.	23	94.3	2.7	69.8	23.0	4.6	2.5	65.8	21.6	4.3
M. M.	21	81.0	3.5	71.0	18.9	6.7	2.8	57.5	15.3	5.4
M. S.	21	99.5	2.9	69.6	22.7	4.9	2.9	69.2	22.6	4.9
B. B	20	95.0	3.3	68.3	21.4	7.0	3.1	64.9	20.4	6.7
C. C.	21	86.8	2.9	74.1	18.5	4.5	2.5	64.3	16.1	3.9
Y J	21	89.9	2.5	69.7	19.5	8.5	2.3	62.6	17.4	7.7
B J	20	65.1	2.7	70.4	20.3	6.6	1.8	45.8	13.2	4.3
Mean	22	87.5	3.0	69.9	21.2	5.9	2.6	61.2	18.6	5.2
P	Not significant	<0.001	<0.001	<0.02	Not significant	<0.001	<0.01	<0.001	<0.001	<0.001

Symbols as in table I

P refers to the significance of the difference between means in the two groups.

Identification of the spots

The four main phosphorus-containing spots showed the same mobility as the lysolecithin, sphingomyelin, lecithin and phosphatidylethanolamine mentioned in Material and methods For further identification colour reactions were used.

The only ninhydrin-positive spot was that identified as phosphatidylethanolamine. In addition sometimes a very faint ninhydrin colour was seen between sphingomyelin and lecithin The ninhydrin reaction was not however routinely used and this spot which has the same mobility as lysophosphatidyl ethanolamine would thus be included with one of these two spots. The lysolecithin, sphingomyelin and lecithin spots gave a positive Dragendorff reaction (39) Sphingomyelin was found to have a tendency to separate in two spots. This observation was also made by Habermann et al. (11). According to Svennerholm (personal communication) this phenomenon is due to different mobility of sphingomyelins with different fatty acid chain length. Fig 1 shows the typical pattern.

As a rule iodine vapour was used for detection of the spots (19) To ensure that all phospholipid spots were made visible by this technique some plates were treated by spraying with sulphuric acid and heating (19) As no further spots were revealed in this way the more convenient iodine vapour was used routinely

In addition to these main spots a faint spot was regularly observed between the start and the lysolecithin spot. It gave a positive reaction with ammoniacal silver nitrate solution (34) and it possibly represents phosphatidylinositol. This component was also eluted from silicic acid and aluminium oxide columns in about the same way as phosphatidylinositol (12). Its phosphorus content was not routinely determined as it was found to give an absorbancy only slightly exceeding that of the corresponding blank area. It thus accounts for less than 1 % of the applied phosphorus. Sometimes a faint iodine colour was given by the start area but not more than 1–2 % of the applied phosphorus remained at the origin. It probably represents water-soluble phosphorus-containing contaminants which have not been fully removed by the washing procedure.

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Robinson and Phillips (31) have also described a method for the determination of serum phospholipids by thin-layer chromatography. These authors after scraping off the

Table IV Average normal values of blood phospholipids

Author	Material	Method	% of total phospholipids				Total lipid phosphorus (µg/ml)	No. of subjects
			PE	Lec	Sph	LL		
Phillips (28)	Serum	Silicic acid column	5	68	19	8	100	15
Etienne & Polonovski (6)	Serum	Silicic acid column	8	61	21	10	88	7
Gjone et al. (9)	Serum	Silicic acid column	6	66	18	9	93	8
Marinetti et al. (21)	Plasma	Silicic acid paper	2	68	21	9	57	2
Vogel et al. (38)	Serum	Silicic acid paper	3	68	19	10	112	4
McArdle & Zilkha (22)	Plasma	Silicic acid paper	3	65	21	11	—	6
Robinson & Phillips (31)	Serum	Thin-layer chromatography	4	66	20	10	83	8
Doizaki & Zieve (5)	Serum	Thin-layer chromatography	3	66	23	10	115	7
Present study	Plasma	Thin-layer chromatography	3	70	21	6	88	9

Includes phosphatidylinositol.
Includes several unidentified substances.

Symbols as in table I

phospholipid spots hydrolysed the phospholipids with perchloric acid filtered off the silica gel and made the colorimetric reaction. This process seems more time consuming than direct phosphorus determination.

Application of the method

As an example of the use of this method values are reported here from a preliminary investigation on the phospholipids during pregnancy Blood was taken in the post absorptive state from five healthy pregnant women during the last month of uncomplicated pregnancy The control group was nine healthy young women (medical students and blood donors) The results are presented in table II

The pregnant women show an increase of total phospholipids. The relative composition is changed Most marked is the decrease of lysolecithin (which is also absolute) Lecithin, and especially phosphatidylethanolamine, show a relative increase The percentage of sphingomyelin is unchanged although there is an absolute increase.

The errors of the method were calculated from the 14 quadruplicate determinations and are presented in table III

Discussion

The described method seems well suited for the determination of the phospholipid composition in plasma. The normal values are in general agreement with those found in the recent literature (table IV) except that the lysolecithin percentage is lower. This is to a slight extent due to loss of lysolecithin in the washing procedure but it may also partly depend on rigorous measures to exclude conversion of lecithin to lysolecithin. Possibly it may also represent a sex difference as some investigated healthy males have shown somewhat higher values. If endocrine factors are active there may be a variation in the phospholipid composition during the menstrual cycle (as has been found for total phospholipids) (27) but this has not yet been investigated. It will be the subject of further study

The values reported here show a marked difference in the composition of the phospholipids in pregnancy as compared with non-pregnant women It is well known that during pregnancy there is a pronounced increase of all the lipid fractions (2 4) inluding the phospholipids, as has also been found here. The only study found in the literature on the composition of the phospholipids during pregnancy is a paper by Helmy and Hack (14) where on paper chromatograms the cephalin" spot seemed rather large in the mother s blood compared to that of the baby s (only visual comparison) In the present study there also was found a substantial increase in the phosphatidylethanolamine value during pregnancy The most pronounced alteration however was the decrease of lysolecithin, both relative and absolute. There was a elative increase of lecithin while the sphingomyelin increase was absolute with no change in the relative amount.

The mechanism of the hyperlipemia of pregnancy is unknown but it is generally supposed that endocrine influences are responsible (4) Only speculations can be made on the cause of the changed phospholipid composition. Popják and Beeckmans (29) have shown that the rabbit placenta very actively takes up phospholipids and possibly this uptake may be selective. The altered phospholipid composition during pregnancy may have a functional role. This is suggested by an investigation where it was found that intravenous administration of phospholipids to pregnant rabbits sensitized them to oxytocin (18) This sensitization could be offset by progesterone treatment.

Only a few recent investigations have been made on the phospholipid composition in blood during various states. Low values for lysolecithin have been found only in serious liver disease (28) and some cases of myocardial infarction (21) The hyperlipemia of nephrosis usually shows high lysolecithin values (26) Other hyperlipemias have not been investigated.

Summary

A method is described for quantitative determination of the plasma phospholipid composition by thin-layer chromatography Special precautions were taken to ensure accurate determination of lysolecithin. Values are reported for nine healthy young women and for five women during the last month of normal pregnancy In the pregnant women the absolute quantity of lysolecithin was decreased while the other phospholipids were increased. Expressed as per cent of total phospholipids there was a decrease of lysolecithin, an increase of lecithin and phosphatidylethanolamine and no change of sphingomyelin.

Acknowledgements

Excellent technical assistance was given by Mrs. Marianne Wetterlin. The study was aided by grants from the Medical Faculty, University of Göteborg and the Swedish National Association against Heart and Chest Diseases.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Oesophageal Moniliasis in Malignant Neoplastic Disease

By

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The occurrence of moniliasis in malignant neoplastic diseases has been the object of considerable interest in the last decade (3 5, 6, 9 10)

Among the various forms of moniliasis the localisation in the oesophagus is of special significance, because this form is more frequently seen than generally assumed, and because pronounced clinical symptoms are often present. It is important to diagnose this disease, as it is possible to treat it successfully with Amphotericin B.

In the following the results of an investigation concerning the frequency of oesophageal moniliasis are presented. All cases occurred in patients who were admitted to the wards of the Cancer Hospital for Jutland, where patients with leukaemia or other malignant systemic diseases were treated.

Material and methods

F om May 1956 to December 1961 694 patients wer admitted. Among these, 98 patients suffered from moniliasis in the oral ca ity or elsewhere (14 %) and 35 patients from moniliasis of the oesophagus (5 %)

Submitted for publication September 11 1963

Among these 35 patients, 30 had only one episode, 4 had two, and 1 had 3 episodes of oesophageal moniliasis.

The main diagnoses are reported in table I.

In the series there were 26 men and 9 women, and even if the sex-distribution of the various main diseases is taken into consideration, there is a considerable prevalence of males.

As can be seen from table II there were patients of all age groups.

The diagnosis of oesophageal moniliasis was made by means of oesophagoscopy in 33 cases and at the same time roentgenographical examination of the oesophagus was done in 32 cases.

The results of the oesophagoscopy and the X-ray examination were compatible in 24 cases. In 8 cases where the diagnoses were obtained by oesophagoscopy the X-ray ex aminations showed a normal oesophagus.

Cultures from the oesophagus were obtained in 37 instances and classification of the isolated yeasts made according to Lodder and Kreger van Rij (7) The results of cultures from the oesophagus are shown in table III

As can be seen candida albicans alone was isolated in most cases. I three cases other yeasts were also found, and in one case torulopsis glabrata seemed to be the only cause of the moniliasis.

In 21 cases the general condition of the patients was very poor In 9 cases the condition

treatment was omitted without any harm to the patients.

Amphotericin B was administered intravenously as drip infusion over a period of at least 6 hours. The daily dose of 12–50 mg was dissolved in 500–1,000 ml of 5 % glucose solution. The initial dose was in all cases about 1/4 mg/kg body weight. Thereafter the daily doses were gradually increased over few days. The final daily dose was in most cases 1/2–3/4 mg/kg body weight, and only few patients received as much as 1 mg/kg body weight. If significant side effects occurred the dose was again slightly reduced. The duration of treatment was in most cases 1–2 weeks, and only one patient was treated for more than a month. The total dose given varied between 80 and 2,760 mg.

Serum creatinine determinations were made before and once or twice weekly during treatment with Amphotericin B. Liver function tests were only made in a few cases. The patients were under close observation for side effects, such as rigor and fever, nausea, vomiting, loss of appetite, general malaise etc.

The effect of treatment with Amphotericin B in 23 courses was beneficial. Sixteen cases showed a definite improvement, 3 cases were classed as satisfactory and only in 4 cases was there no effect. The remaining 6 courses were of very short duration (4 days or less) and were considered insufficient in evaluating the effect.

Rigors, fever and/or local reactions (thrombophlebitis) at injection sites were observed in 9 instances. In no case did the side effects indicate discontinuation of treatment although in some cases we found it necessary to withdraw treatment for one or two days.

No side effects of serious nature were observed and particularly no increase in serum creatinine has been found in any case.

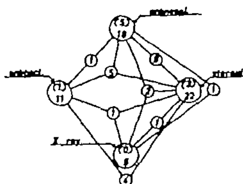


Fig. 1 Treatment prior to oesophageal moniliasis. The figures in brackets indicate the number of patients given the treatment concerned alone. The other figures in the large circles indicate the number of patients receiving combined therapy and the combinations are indicated by means of the various lines.

Discussion

It has been shown that oesophageal moniliasis occurs quite often in patients with systemic malignant disorders. The frequency found seems to be higher than that presumed earlier and is in agreement with the results reported by Gruhn and Sanson (5) of an autopsy material from patients suffering from the same category of diseases.

It is difficult to decide whether an increase in the frequency of oesophageal moniliasis has taken place, as we do not know of previous investigations comparable to ours.

The modern therapy with antineoplastic and antibacterial agents, steroids and X-ray is often and justly thought to be responsible for the increase of secondary infections especially in patients with lowered resistance to bacterial and mycotic diseases, e. g. chronically ill patients, patients with various malignant disorders of the blood etc. (4)

In fig. 1 we have shown the therapeutic combinations used for the main disease. As all the patients were treated with one or several of the abovementioned therapies it has not been possible to show a closer relationship between the appearance of the moniliasis and any specific form of therapy.

Table I Main diagnoses of cases with oesophageal moniliasis

Disease	No.
Acute leukaemia	10
Hodgkin's disease	8
Myeloid leukaemia	7
Lymphatic leukaemia	5
Reticulosarcoma	4
Myeloma	1

Table II Age distribution of 35 patients with oesophageal moniliasis

Years	No.
0–10	1
10–20	2
20–30	9
30–40	4
40–50	3
50–60	5
60–70	7
70–80	3
80–90	1

Table III Yeasts found in cultures from the oesophagus in 35 patients with moniliasis

	No.
C. albicans	33
Torulopsis glabrata	1
C. albicans and C. krusei	1
C. albicans and C. tropicalis	1
C. albicans, C. tropicalis and Torulopsis glabrata	1
No culture mad	4

Table IV Main symptoms of oesophageal moniliasis

	No.
Pain on swallowing	19
Obstruction of food passage	17
"Lump in the throat	5
Retrosternal pains	3
Dryness in the throat	2

was poor in 7 it was fair and in only 4 cases was it good

All except 7 had an increased sedimentation rate, and all except 6 were anaemic. Thirty patients had lymphocytopenia while 22 had a granulocytopenia. In 3 cases the gamma-globulin values were found to be below the normal limits.

The question arises, at which time during the course of the main disease did the patients acquire their mycotic oesophageal infection. In 14 cases the patients had been ill for less than 1 year before the appearance of the yeast infection, whereas in 10 cases they had been ill for 1–2 years, and in 5 cases 3–10 years. The cases were distributed evenly throughout the year with no apparent seasonal variation.

The patients were treated with various combinations of antibacterial and antineoplastic drugs and corticosteroids. Some patients also received X ray treatment.

In an attempt to get a more detailed view of the possible relationship between treatment and the appearance of the moniliasis, we have in fig 1 illustrated the various treatments which the patients have had during the last month before the appearance of their oesophageal moniliasis.

In addition most of the patients have had the various forms of treatment during the last year and most of them were treated in the same way while suffering from their moniliasis.

The majority of the patients had very disagreeable symptoms caused by the mycotic infection, those most commonly seen being given in table IV

Treatment with Amphotericin B

Twenty three patients were treated with Amphotericin B. A few of them had two or three attacks of moniliasis and were treated in each of the attacks so that a total of 29 courses of Amphotericin B were given.

Three patients received no Amphotericin B but had Nystatin or Pentamidine The remaining 9 patients were given no antimycotic therapy at all. In five of these signs of oesophageal moniliasis were not found until just before death or at autopsy The last four cases had such slight symptoms from their moniliasis and their general condition was so good or was improving so quickly that

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We found it of interest to compare the roentgenographical picture with oesophagoscopy. The roentgenographical picture has been described by Andrén and Theander in 1956 (1). Irregular and ragged outlines of the oesophagus with numerous indentations and protusions were found in most cases where oesophagoscopy showed moniliasis.

In some cases the roentgenographical picture was normal despite pathological findings in oesophagoscopy. Consequently it is clear that oesophagoscopy yields more valuable diagnostic results, although it is somewhat more distressing for the patients.

In view of this circumstance it is worth noting that even though the roentgenographical picture is not specific the finding of moniliasis in the oral cavity in combination with a pathological X-ray picture is nearly conclusive as to the diagnosis.

It is difficult to evaluate the results of Amphotericin B therapy. This is due to the fact that in some cases an improvement occurs which may be caused by the treatment of the main disease leading to a general improvement which may increase the patient's resistance to mycotic infection.

However, we feel certain that the antimycotic treatment is beneficial, as we have observed cases in which the moniliasis disappeared without any improvement of the main disease.

It should be noted that cure has often been obtained in our series by means of smaller doses and shorter duration of treatment than used in the therapy of mycoses other than moniliasis (2). Thus we have often seen complete relief of even severe subjective symptoms in the course of 2 days treatment.

We have some experience with other forms of antimycotic therapy including Nystatin and Pentamidine. The former does not seem to have any effect at all, while the latter has been successfully used in some cases (8). The side effects of Pentamidine are, however, so frequent that Amphotericin B should be preferred.

It is true that the patients very often acquire their oesophageal moniliasis sub finem and this fact may make the treatment seem unjustifiable. However, we want to emphasize that because the clinical symptoms of this complication are so troublesome the treatment, even in such circumstances, is usually well indicated.

Summary

Among 694 patients with leukaemia and other malignant systemic disorders treated over a period of 5 years 14 % had moniliasis in the oral cavity or elsewhere and 35 patients (5) suffered from oesophageal moniliasis. X-ray examination of the oesophagus was made in 32 cases and oesophagoscopy in 33 cases. The symptomatology and mycology in this condition is presented. Treatment with Amphotericin B was beneficial in many cases.

Acknowledgement

Aided by grants from the Anders Hasselbalch Anti-leukaemia Foundation, Carl Schepler and Wife's Bequest, the Irma Foundation and Statens Almindelige Videnskabsfond.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Uricosuric Effect of Dicoumarol

By

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It has been known for some time that certain drugs, which are chemically coumarins and indandiones, and which are used clinically as anticoagulants, produce a uricosuric effect in man. It has thus been demonstrated that several of these anticoagulants on administration to human beings give rise to an increase in the urinary excretion of uric acid and a decrease in the plasma concentration of uric acid.

Such a uricosuric effect of a coumarin preparation was first described by Sougin-Mibashan and Horwitz (11) after administration of ethyl biscoumacetate (3,3' carboxyethylmethylene -bis (4 hydroxy coumarin) Tromexan®) in human beings, and this effect has since been confirmed by Ogryzlo and Harrison (8) and Thompson et al. (12). A similar uricosuric effect of dicoumarol (3,3'-methylene-bis-(4-hydroxycoumarin)) was reported by Hansen and Holten (6) and Dreyfuss and Czaczkes (4). Thompson et al. performed a comparative study of the uricosuric effect of four different coumarin and indandione drugs. They administered the drugs in a dosage such that the effects as assessed on the basis of blood-coagulation studies were nearly identical. Administered in this way ethyl biscoumacetate and, to a lesser degree, phenylindandione (2 phenyl-indandione 1,3) had a pronounced uricosuric effect. On the other hand, the two other drugs studied — acenocoumarin (3-(α-p-nitrophenyl β-acetyl-ethyl)-4 hydroxycoumarin, Sintrom®) and anisindione (2-(p-methoxy phenyl)-indandione-1,3 Miradon®) — were without demonstrable uricosuric effect. Thus, no evidence was disclosed in favour of the assumption that the uricosuric action varies in parallel with the anticoagulant action. Pasero (9) also reported that no correlation seems to exist between the uricosuric and anticoagulant actions of coumarin drugs.

The mechanism of the uricosuric action of anticoagulants is largely unknown. In the light of the results of the aforementioned studies by Sougin-Mibashan and Horwitz and Thompson et al., it is, however, reasonable to assume that the effect is due to a decreased re

Submitted for publication September 11, 1963.

dicoumarol from acidified plasma or urine by means of n-heptane. The substance is re-extracted from this by 2.5 N NaOH. Th content of dicoumarol, which has a distinct absorption maximum at a wavelength of 315 mμ is then determined spectrophotometrically. It is reported that the presence of dicoumarol metabolites in the plasma does not interfere with th determination. In the present study this method was used with th slight modification that the acidification of the plasma was done with 1 N HCl instead of with 3 N HCl as originally stated. With this slightly modified technique number of experiments were performed in which the recovery of dicoumarol added to human plasma in amounts ranging from 10 to 40 μg/ml was studied. The mean recovery rate obtained was 97.9 % (n = 8) and the standard error of this mean was calculated as ± 1 %. The results of the plasma-dicoumarol determinations given are not corrected for this incomplete recovery. All dicoumarol determinations were performed in duplicate, and the mean value of the two determinations was taken as the result. The individual determinations rarely differed more than 4 % from the mean value.

The dicoumarol determinations on urine were performed according to the same method as was used in the plasma-dicoumarol determinations. The specificity of dicoumarol determinations in urine seems to be lower than in plasma, since urine samples from untreated persons revealed appreciable amounts of apparent dicoumarol. This fact coupled with the low concentration of dicoumarol in the urine of dicoumarol-treated persons rendered the determinations on urine fairly uncertain.

Uric acid in the plasma and urine was estimated by the uricase method described by Praetorius and Poulsen (10). The results are given as the mean value of duplicate determinations. The individual determinations rarely differed more than 2 from the mean alue

Creatinine in the plasma and urine was determined by the method of Owen et al. (7). Experiments with recovery of creatinine added to human plasma in amounts from 5 to 15 μg/ml showed mean recovery rate of 98.0 (= 6). The standard error of this mean was found to be ± 0.9 %.

Table I. Urinary excretion of uric acid in μg/min/1.73 sq.m body surface in the four subjects A, B C and D before and after oral administration of dicoumarol

Periods (hours after ingestion)	A	B	C	D	Mean values
Control	200	252	320	371	286
2—4	340	250	—	—	—
6—8	515	427	504	576	506
11—13	481	624	389	393	472
23—25	683	559	549	574	641
31—33	567	345	635	405	488
47—49	394	329	372	584	370
55—57	451	306	—	—	—
71—73	234	310	502	544	363
119—121	—	244	429	378	(350)
167—169	—	—	333	—	—

Results

The results of the experiments appear from fig 1 and from table I. It should be noted that the results from the control periods are extrapolated to zero time, and that the values for the uric acid clearances and urinary excretion of uric acid are indicated by dots corresponding to the middle of the individual periods.

In the periods following administration of dicoumarol, the urinary excretion of uric acid showed increasing values (table I). The maximum values for the urinary excretion of uric acid were found in the four experiments from about 12 to 36 hours after the administration, and were from slightly less than two to about four times as high as the values recorded for the corresponding control periods. Thus, while the average excretion of uric acid in the control periods was found to be 286 μg/min the four subjects showed an average maximum excretion of 641 μg/min. 24 hours after the administration of

absorption or an increased secretion of uric acid by the renal tubules and not to an enhanced filtration rate in the glomeruli It is uncertain if the effect on the kidneys is referable to an anti vitamin K effect Pasero mentioned a number of factors which weigh against this explanation

Apart from the inhibitory action on blood coagulation anticoagulant drugs of the coumarin or indandione group exert few pharmacodynamic effects on the organism. The uricosuric action must be included among these effects At the present time it appears doubtful if this effect of anticoagulants is of any significance in connexion with their therapeutic use. Both Sougin Mibashan & Horwitz and Hansen & Holten have however suggested such a possibility

Thus the uricosuric effect of anticoagulants seems, for several reasons to be of interest, and further studies of this effect may contribute to our understanding of the mode of action of these drugs in the organism

The present paper is a report of the results of an investigation on the uricosuric action of dicoumarol as determined by clearance studies in four subjects. In addition attempts were made to throw light on the relationship of this action to the plasma concentration and urinary excretion of dicoumarol

Material and methods

The investigation reported was performed on four men admitted to the First University Clinic of Internal Medicine, Aarhus Kommunehospital. The four subjects, who are hereafter referred to as A B, C and D presented no clinical signs of hepatic, renal or cardiac disease. Furthermore, none of the subjects studied had any known predispositions to disorders of uric acid metabolism.

Before and during the experiments, the subjects were given an ordinary hospital diet, and during the experimental periods no changes occurred in their daily activities, except those necessitated by the experiments. The clearance studies were performed by collection of 2-hour urinary samples and blood samples were withdrawn exactly in the middle of each 2 hour period. In all the experiments, spontaneously voided urine was used, and the subjects had been told beforehand that it was important that the evacuation of the bladder should be as complete as possible. In order to ensure a suitable urinary output during the individual clearance periods, the subjects ingested at least 0.5 l of water during the first half hour of each period. After measurement of the volume, the total urinary specimen was stored at − 20 C until the analysis was performed The heparinised blood samples were immediately centrifuged, and the plasma was pipetted off. The urine and plasma were analysed for their content of uric acid, creatinine and dicoumarol. However dicoumarol determinations were performed only on the urine from B and C. The clearance values for uric acid and creatinine were calculated in the usual way and the values obtained were corrected to a body surface of 1 73 sq.m. The body surfaces of the subjects were determined by the use of a nomogram based on Du Bois height weight formula (2).

The first clearance period – early in the morning of the first day – was used as a control period. Immediately after the end of the control period each subject was given a single oral dose of dicoumarol. The doses were 400 mg in A and B and 500 mg in C and D Dicoumarol was invariably given in the form of 50 mg tablets.

The clearance studies were performed at suitable intervals during the period immediately following the administration of dicoumarol. These studies were continued until the plasma concentration of dicoumarol had reached a suitably low level, usually for 120 hours. However in subject A, the experiment was interrupted after the lapse of 72 hours.

The plasma concentration of dicoumarol was assessed by means of ultraviolet spectrophotometry by the method of Axelrod et al. (1) This method is based upon extraction of

dicoumarol. At 72 hours the average excretion of uric acid had fallen to 363 μg/min.

The plasma concentrations of uric acid in the four subjects tended to fall after administration of dicoumarol. In three of them, the fall was most pronounced at about 32 hours after the administration, while the minimum occurred as early as at 12 hours in the fourth (D). The mean value of the plasma concentration of uric acid in the control periods was found to be 56.0 μg/ml at 32 hours after the administration of dicoumarol it was 45.0 μg/ml, and at the end of the experiments it had increased to 53.8 μg/ml.

The values of the uric acid clearances increased during the first 12 to 36 hours after administration of dicoumarol, after which time decreasing values were obtained. During the control periods before the administration of dicoumarol, the mean value of the uric acid clearances was 5.1 ml/min, while the average of the maximum values recorded was 14.1 ml/min. At 24 hours the mean value of the uric acid clearances reached maximum of 11.6 ml/min and at the end of the experiments the mean value recorded was 5.8 ml/min.

The uric acid/creatinine clearance ratio also showed an increase after dicoumarol administration. Peak values were recorded during the period from 12 to 36 hours after administration. For the control periods this ratio expressed as percentage averaged 6.2, while the average of the maximum values recorded was 13.8. At 24 hours the mean value was 10.2 at 32 hours 10.8 and at the end of the experiments 6.8.

In the four subjects, the plasma concentration of dicoumarol was found to reach a maximum within the first 12 hours after the administration of dicoumarol. The peak concentrations of dicoumarol observed in A, B and C ranged from about 26 to about 21 μg/ml while D who also revealed the least uricosuric effect after dicoumarol, showed a peak concentration of only 16.9 μg/ml. When the maximum plasma concentration of dicoumarol had been recorded, the concentrations decreased with time as in a first-order reaction. After the lapse of 120 hours, the plasma concentration of dicoumarol had fallen to between 1 and 2 μg/ml in the subjects B, C and D. As already stated the experiment was interrupted in A at 72 hours after administration at this time the plasma concentration of dicoumarol was still about 5 μg/ml.

The urinary excretion of dicoumarol, which was studied in all periods in the subjects B and C, was found to be very low. The maximum excretion demonstrated amounted to approx. 1 μg/min. and the total excretion was calculated as less than 1 of the dose administered.

By comparing the plasma dicoumarol concentrations in the individual cases (A, B, C and D) with the corresponding values of uric acid clearances, the ratios of uric acid clearance to creatinine clearance and with the plasma uric acid, it is evident that no simple relationship (i. e. proportionality) exists between the plasma dicoumarol concentration and the uricosuric action measured by these quantities. It should be noted that the maximum concentration of dicoumarol in cases A and C was observed at least 12 hours before the maximum uricosuric effect was obtained. It should also be noted that there did not seem to be any definite correlation between the urinary excretion of dicoumarol and the observed uricosuric effect.

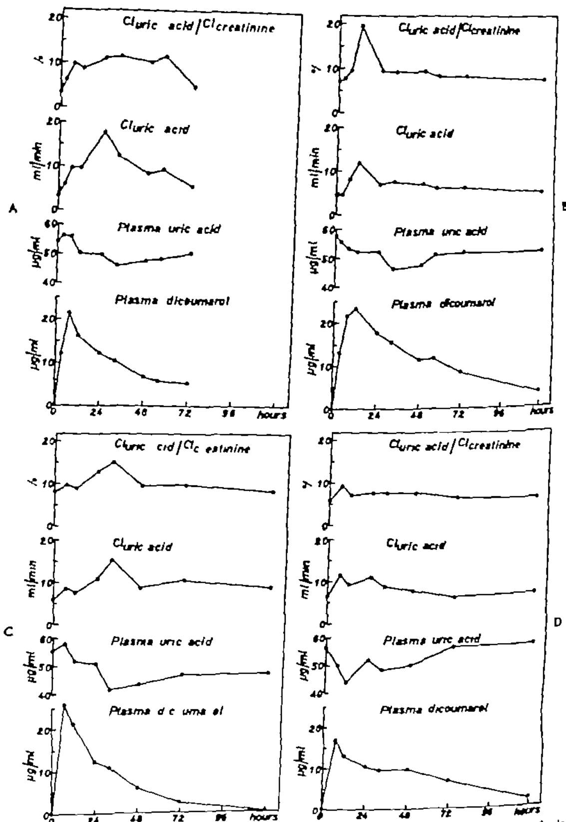


Fig 1 The concentrations of dicoumarol and uric acid in the plasma and the clearance of uric acid after a single oral dose of dicoumarol (400—500 mg) given to each of the subjects A, B, C and D. The ratios of uric acid clearance to creatinine clearance are also given. The values for uric acid clearance are calculated for a surface area of 1 73 sq.m.

uric acid of 2.0 mg/100 ml, and after oral administration of 350 mg phenyl indandione a similar average fall of 1.5 mg/100 ml. Judging from these figures, therapeutic doses of dicoumarol seem to have a somewhat lower uricosuric effect than corresponding doses of ethyl biscoumacetate and an effect of almost the same magnitude as therapeutic doses of phenylindandione.

The decrease in the plasma level of uric acid is presumably due to the increased urinary excretion. On the basis of the present investigation it cannot be decided if other factors also contribute to the induction of this effect.

The mechanism of the uricosuric action of dicoumarol and other anticoagulants is at present unknown. The increase in the ratio of uric acid clearance to creatinine clearance observed in this study after ingestion of dicoumarol and also described after ingestion of other anticoagulants (11 12) indicates a tubular mechanism as a cause of the uricosuric action. An increase in the amount of uric acid filtered by the renal glomeruli might contribute to this effect. As the amount of uric acid filtered in the glomeruli is equal to the product of the concentration of filterable uric acid in the plasma and the rat of glomerular filtration it is evident that this amount would increase if one of the aforementioned factors increased. It is widely accepted ew that all the uric acid in the plasma is freely filterable in the glomeruli (5 15). An increase in the filtered amount of uric acid should then be possible only if the rate of glomerular filtration increased. On the assumption that the size of creatinine clearance reflects this filtration rate, it is possible from the present study to judg the significance of this factor. After ingestion of dicoumarol the mean values for creatinine clearance in the various periods showed a certain but low increase (5—15 %) in comparison with the value for the control periods. In one period a somewhat higher value (about 30 %) was observed. From this it may be concluded that the uricosuric action of dicoumarol is mainly or purely of tubular origin, and that increased filtration by the glomeruli is of only minor importance, if of any. On the basis of the studies of Gutman et al. (5) and others it may be said that it is highly probable that uric acid is both reabsorbed and secreted by the tubular cells. Dicoumarol must exert its uricosuric action through one of these factors, but at present it is not clear which of these is of importance. Among the various possibilities of the mechanism of action of dicoumarol on the renal tubules two will be mentioned. First, the possibility that dicoumarol and uric acid should compete for a common system of transport in the tubules seems unlikely as it is known that dicoumarol is not filtered by the glomeruli because of extensive binding to the plasma proteins especially the albumin fraction (13) and as stated, no excretion of unchanged di coumarol occurs in the urine. Secondly the possibility that dicoumarol in the organism might be metabolised to a uricosurically active substance should be mentioned. Such a metabolic process would be assumed to be time-consuming and a certain time lag between maximal dicoumarol concentration in the plasma and maximal uricosuric action observed should be expected. Such an observation was actually made in experiments A and C. However as the metabolic transformation of dicoumarol in man is unknown (14) it is not possible to test this hypothesis further at present. The observation made by Burns et al. (3) that a

Discussion

From the results reported above it appears that the administration of di coumarol in man affects the urinary ex cretion of uric acid After oral administration of the drug in doses ranging from 400 to 500 mg the urinary excretion rose to a mean maximum value of about twice the value recorded in the control period The uric acid clearance also showed an increase which was almost of the same order of magnitude. In comparison it may be stated that Hansen and Holten (6) determined the urinary ex cretion of uric acid during 24-hour periods after oral administration of 500 mg dicoumarol in two persons In these experiments, they observed an increase in the clearance of uric acid of 45 and 31 % as compared with the preceding control periods. However these figures cannot be directly compared with the results re ported in this paper because of the dif ference in the lengths of the clearance periods used in the two studies. If this difference is taken into account, the results obtained do not seem to be di vergent.

In order to place dicoumarol among other uricosuric anticoagulants the present results may be compared with those obtained by others in studies on the uricosuric effect of ethyl biscoumacetate and phenylindandione Sougin Mibashan and Horwitz (11) found in two persons, after oral administration of 1 8 and 1 5 g ethyl biscoumacetate an increase in the uric acid clearance to 6 and 8 times the values recorded during the preceding control periods. In these experiments, short clearance periods of about 30 minutes were used Thompson et al (12) found in four subjects, who had each ingested 2 000 mg ethyl biscoumacetate, an increase in the uric acid clearance. The mean value of the recorded maximum uric acid clearance here was 4 times greater than the mean value during the control periods. After oral administration of 300 mg phenylindandione to each subject, the mean value of the uric acid clearance increased to 1 5 times the value which had been recorded in the preceding control period In both experiments, the clearance periods extended over 3 hours. The doses of ethyl biscoumacetate and phenylindandione just mentioned and those of dicoumarol used in the present study are frequently used as therapeutic initial doses of these anticoagulants. As a rule, these doses will exert roughly the same effect on blood coagulation. When the uricosuric action of these three anticoagulants is compared on the basis of these doses, it is seen that the effect of ethyl biscoumacetate is considerably greater than those of phenylindandione and dicoumarol while the latter two seem to have approximately the same effect. If the size of the doses administered is taken into account the difference in the uricosuric action of the three anticoagulants is less pronounced.

As stated in the section on the results, a fall in the plasma level of uric acid was observed after administration of dicoumarol. On an average, this fall amounted to 11 0 μg/ml after the lapse of 32 hours as compared with the mean value of the plasma uric acid in the control periods. In comparison, it may be stated that Hansen and Holten found an average fall in the serum level of uric acid of 1 8 mg/100 ml after oral administration of di coumarol in doses ranging from 500 to 700 mg After oral administration of 2,000 mg ethyl biscoumacetate to four persons, Thompson et al. observed an average decrease in the serum level of

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Malabsorption of Vitamin B_{12} During Treatment with Para-aminosalicylic Acid

A Preliminary Report

By

O. Heinivaara and I. P. Palva

Para-aminosalicylic acid (PAS) is known to have caused e. g. such haematological complications as haemolytic or aplastic anaemia, leukopenia, agranulocytosis and thrombocytopenia (5). We have not found in the literature, however, any report of PAS-induced megaloblastic anaemia. We recently established two cases of this type of anaemia which had developed during PAS therapy. These were not cases of Addisonian pernicious anaemia as the intrinsic factor (IF) did not correct the absorption deficiency of vitamin B_{12} demonstrated by the Schilling test. On the contrary, the Schilling test values after the discontinuance of PAS, even without IF, were normal. Because of the likelihood that PAS might in these cases have had a role in the origin of megaloblastic anaemia, we decided to study the effect of PAS on the absorption of vitamin B_{12}.

Material and methods

Our series consisted of 10 patients taking PAS for pulmonary tuberculosis. The Schilling test was performed on a number of the patients before the inception of the PAS regimen and again after some weeks of the therapy. The tests were done in the reverse order on the rest of the patients: initially during long term PAS therapy and a second time a few weeks after the termination of therapy. The patients received other antituberculous drugs concurrently, but the only medicine added or withdrawn during the test period was PAS.

The Schilling test was performed according to Schilling (4) using 0.7 μg of vitamin B_{12} ^{60}Co (Abbott lab.). In the Schilling II test, one capsule of purified IF preparation (Abbott lab.) was used as the additive.

Submitted for publication September 26, 1963.

Results

Table I shows the Schilling values of all the patients during PAS therapy and without PAS, and the Schilling II values during PAS for 5 patients. The Schilling value without PAS was at least 5 times the value obtained during PAS therapy in 7 cases and the difference was 2—3-fold in the other 3 cases. Although the values were not distinctly pathological (under 5 %) during PAS therapy in all the cases, the trend of the changes was the same in

metabolite of a phenylbutazon analogue (G-26671) showed a much more pronounced uricosuric effect than the parent drug indicates that such a mechanism would not be unreasonable

Some problems concerning the mechanism of the uricosuric action are discussed The possibility that the uricosuric action of dicoumarol might be ascribed to a metabolite of this drug is mentioned.

Summary

The uricosuric effect of a single oral dose of dicoumarol (400—500 mg) was studied in four subjects Urinary excretion of uric acid uric acid clearance and the ratio of uric acid clearance to creatinine clearance all showed increasing values after ingestion of dicoumarol. Maximum mean values were obtained after the lapse of about 24 hours and were about twice the mean values obtained in the control periods The plasma uric acid concentration decreased to a minimum at about 32 hours after the administration of dicoumarol The average decrease was 11 μg/ml No simple relationship was found between the dicoumarol concentration in the plasma, which showed a maximum value at 8—12 hours after dicoumarol ingestion and the uricosuric effect, but nevertheless a certain relationship between the maximum concentration of dicoumarol in the plasma and the maximum uricosuric effect may exist The urinary excretion of dicoumarol was studied in two persons and was found to be very low No relationship between urinary dicoumarol excretion and uricosuric action was established

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chilling values during PAS therapy were change in storage or in renal excretion, reater quantities of vitamin B_{12} than mal should be retained in the organism. Our 2 cases of megaloblastic anaemia which developed during PAS therapy argue, however for a vitamin B_{12} deficiency in the organism. We therefore regard it as justifiable to interpret the lowered Schilling values during PAS therapy as due to impairment of absorption caused expressly by PAS.

Although PAS is known to cause gastric distress very frequently the B_{12} absorption disturbance demonstrated can hardly be due to a possible PAS-induced gastritis and reduced secretion of IF since the addition of IF did not normalise the Schilling values. Some calcium-chelating agents may inhibit the absorption of vitamin B_{12} (1 2) but since PAS does not as far as is known exert any such calcium-chelating effect, even this cannot explain the lowered absorption of vitamin B_{12}. We would imagine that the question concerns an absorption disturbance in the area of the small intestine. More detailed clarification of its character calls, however for further studies.

Although the preliminary series reviewed here is small, lowered absorption of vitamin B_{12} during PAS therapy was demonstrated in every patient. However in spite of the extensive use of PAS megaloblastic anaemia is by no means a common complication of PAS therapy. This is partly because of the fairly considerable vitamin B_{12} stores of the organism it takes a long time for the deficient supply of vitamin B_{12} due to PAS to be manifested as megaloblastic anaemia. Secondly although long-term use of PAS is practised in the treatment of tuberculous its side effects, gastric distress and nausea, make its ingestion unpleasant for many and there may consequently be interruptions in the therapy. According to studies made (3) as many as half of the out patients neglect to take the drug regularly. The third reason for the uncommon occurrence of megaloblastic anaemia may be that the patients are often given various vitamin preparations during PAS therapy which nowadays frequently contain vitamin B_{12} as well. As the daily diet thus contains unusually large quantities of it, it is possible that the organism can, despite impaired absorption obtain the minimum requirement of vitamin B_{12} and thus evade manifest anaemia.

Summary

After observing two cases of megaloblastic anaemia which developed during PAS therapy the authors studied the effect of PAS on the Schilling test values. The Schilling value was lowered during the PAS regimen in all the 10 cases examined. The difference was at least 5-fold in 7 cases. As IF failed to normalise the urinary excretion of radiovitamin B_{12}, it was considered possible that malabsorption of vitamin B_{12} in the area of the small intestine was involved. Its detailed clarification requires further studies.

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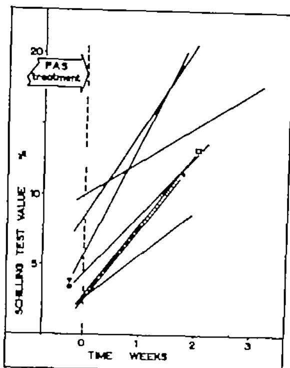


Fig 1 Change of Schilling test values after the cessation of PAS therapy in 7 cases.

Table I Schilling-test values without PAS treatment and during it

Case	Without PAS treatment	During PAS treatment	
		Without IF	With IF
1	19.8	4.8	—
2	9 0	1.8	—
3	21 0	7 0	—
4	18.0	9.4	—
5	11.5	1.5	—
6	20.4	3.8	3.6
7	5.8	2.5	4.8
8	20.0	11.5	8.6
9	24 0	3.4	2.8
10	13.2	1 4	6.6

each case lowered values during PAS medication. The addition of IF did not normalise the Schilling values.

Fig 1 shows the cases in which the test was made during therapy and repeated after the termination of PAS Fig 2 shows finally a case in which it was possible to perform the Schilling test before the institution of PAS therapy and during and after its termination. The figure shows that administration of PAS for roughly a month and a half sufficed to lower the Schilling value to a pathological level but that the change was reversible and the Schilling value returned to normal about 2 weeks after the termination of PAS

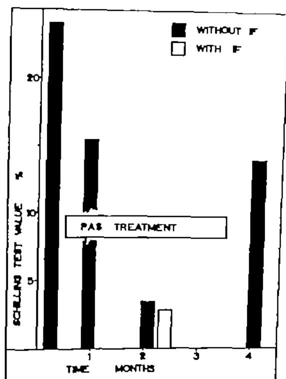


Fig 2 Schilling test values of the patient tested prior to, during and after PAS therapy

Discussion

The method used the Schilling test, naturally does not measure the absorption of vitamin B_1 from the intestine directly The decreased urinary excretion of radioactive cyanocobalamin may indicate a change in the absorption, storage or renal excretion of vitamin B_{12}. However if the reason for the lowered

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Anticoagulant Treatment of Acute Myocardial Infarction

The Importance of Adequate Dosage for the Course of the Disease

By

Lizzie Saxo Strømgren

Opinions on the value of anticoagulant treatment in acute myocardial infarction have become somewhat conflicting.

At an early stage in the history of this treatment, Holten (3) initiated and co-ordinated an investigation of a series of cases derived from 21 medical departments in Denmark. For a two-year period patients admitted to these departments with acute coronary occlusion were divided into two groups depending on whether they were admitted on an even or an odd day. One group was treated with anticoagulants, the other not. Treatment in all other respects being identical, the two groups were comparable.

Independently Wright et al. had started an investigation on the same lines. Their first publication appeared in 1948 (7) and their final comprehensive report (8) was published in 1954. Both these authors and Holten found a significant reduction of the mortality-rate in the treated group as compared with the non-treated group.

In four municipal departments in Copenhagen, over a four-year period, Hilden et al. (2) treated with anticoagulants all patients with acute myocardial infarction admitted to two of the departments while the other two departments used no anticoagulants in cases admitted there. After the lapse of two years the departments changed over and hitherto non-treatment departments started the use of anticoagulants and the others stopped the use of these drugs.

Whereas the first mentioned authors found a lower mortality rate in the treated group, Hilden et al. (2) came to the conclusion that there was no significant difference between the mortality rates of the treated and the untreated groups.

If one has formed the opinion that anticoagulant treatment of acute myocardial infarction is valuable, the consequence must obviously be that all patients should be given this therapy unless contraindications are present, and a control group is then no longer obtainable.

The possibility of undertaking relevant comparisons in patients treated with anti

Submitted for publication September 30, 1963.

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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coagulants is, however not precluded Patients in whom adequate treatment has been given can be compared with patients in whom the treatment has been inadequate, as evaluated by means of the plasma prothrombin *level achieved* Such a comparison has been made in the *present series It should however be* pointed out that this comparison concerns only the occurrence of those complications for which this treatment has been designed and which with our present knowledge it can be expected to prevent It does not specifically consider the mortality rate. This entails that the group with thrombo-embolic complications, including recurrence cases (re-thrombosis) as well as cases with an extension of the original coronary occlusion during treatment (these cases are here tabulated as *+ thrombo-embolism*) also contains some patients who have survived such complications on the other hand the group of cases in which such complications did *not* occur (tabulated as *0 thrombo-embolism*) includes some patients who died during treatment but whose death could not be ascribed to causes which the treatment is known or believed to be able to prevent

The purpose of anticoagulant therapy in acute myocardial infarction is

1) *to prevent* or hamper the extension of the coronary occlusion which leads to widening of the infarction area

2) to prevent the occurrence of recurrent coronary occlusion,

3) to prevent or to hamper the formation of mural thrombi representing a potential risk of peripheral arterial embolism, and

4) to prevent or to hamper thrombophlebitis and allied conditions especially in the lower limbs and in the pelvic veins, the most common cause of pulmonary embolism and infarction

Material and methods

In the two medical university departments in Aarhus Kommunehospital the case records of patients with acute ischaemic heart disease have been kept in special files arranged in chronological order according to the date of the most recent admission. The year 1960 in these files has been chosen as the source of material for the present investigation.

All anticoagulant-treated patients of the *1960 case material of acute coronary occlusions* have been considered. The series includes one patient who perhaps had no coronary occlusion and another who was given anticoagulant treatment for pulmonary infarction these two patients had some months previously had acute myocardial infarction and had at that time been given anticoagulant *treatment which was resumed at this* admission. All were treated in accordance with the customary principles of these departments. These are chiefly 1) anticoagulant therapy 2) coronary regime

1) *Anticoagulant therapy* comprises intramuscular injections of heparin prolongatum a preparation containing per ml 10,000 I. U *of heparin to which 1.25* carboxymethylcellulose has been added (1 4) This preparation has the advantage that it can readily be sterilized and easily injected with a thin cannula. The dose is based on a rough estimate of body weight. Heavy weights are given 2 ml as a starting dose and 1.5 ml every 8th hour altogether 6 injections, average weights are given an initial injection of 2 ml followed by 1 ml 5 times and light weights are given 1 5 ml initially followed by 1 ml 5 times. *Simultaneously with* the initial dose of heparin, 300—500 mg dicoumarol is given depending on the estimated body weight. Before beginning treatment the prothrombin level is determined so that patients with certain complicating diseases contraindicating this treatment may be excluded before any anticoagulant is given. Subsequent doses *of dicoumarol are based* on daily determinations of the prothrombin index by J Lehmann's modification (1941) of Quick's test (5)

The result obtained is termed the prothrombin index expressed as

$$\frac{\text{clotting time of normal plasma} \times 100}{\text{clotting time of of the patient's plasma}}$$

Thus, the prothrombin index is not the percentage prothrombin content but the reciprocal value of this. It is of utmost importance that the thromboplastin (tissue extract) employed should be active and constant. The tissue extract employed in our laboratory has been produced by the State Serum Institute, Copenhagen, and the clotting time for normal human plasma has constantly been from 15–17 sec. If the normal prothrombin time has been outside these narrow limits the extract has been discarded and a fresh one obtained. Hence the numerator in the fraction from which the 'prothrombin index' is calculated is constant and virtually identical throughout the whole material, and so all indices given in this paper are comparable.

When the plasma prothrombin times are plotted against the percentage content of prothrombin as determined by diluting the plasma with saline or (perhaps better) $BaSO_4$-adsorbed plasma, small difference in *prothrombin – per cent* when the content is low (e. g. change from 10 to 5) corresponds to a great difference in clotting time whilst the same difference of 5 near or in the normal zone (e. g. from 80–75 %) corresponds to quit small difference in clotting time When the index is employed the results in the low range and especially below the therapeutic range show numerically greater difference and this may be of some value in arousing the awareness of an observer with limited experience whereas the seemingly insignificant percentual difference may impress him inadequately In eality there is of course no distinction. The effect is purely psychological, but may still be of value

When prothrombin index determination is carried out according to the conditions just described, a prothrombin index of e. g 50 $\left(\frac{15}{30} \times 100\right)$ corresponds to twice the normal clotting time furthermore an index of 33 means that the patient plasma has a clotting time three times as long as normal plasma and so on. I should be noted that the presence of heparin renders the determination of 'prothrombin content by Quick test unreliable and, since 'heparin prolongation has been administered for 48 hours after commencement of the treatment, the index cannot be valid until the 3rd day The choice of an index value of 50 as the upper limit for adequate treatment (see below) means that we consider a prolongation of the clotting time to about twice the normal as sufficient.

2) *Coronary regime* means strict confinement to bed for at least the first week after the onset. During the first 3 or 4 days only fluid diet is given to void defaecation and distension of the stomach. After this stage the patient is helped by a nurse with feeding and washing After a week this regime is very gradually alleviated. The use of a commode at the bedside for defaecation is usually permitted at an early stage. The diet is according to circumstances gradually expanded. The patient is kept in bed for usually 4 weeks about 2 or 3 weeks after admission mild exercises of the lower extremities are started. Usually discharge takes place after 5–6 weeks.

After treatment for bout 4 weeks dicoumarol is either discontinued or gradually decreased, unless long-term treatment has been decided upon. This is the programme adopted for cases running smooth course, but it may have to be varied in the individual case.

The main condition determining the inclusion of the cases of myocardial infarction in this series is that anticoagulant therapy was given during the year 1960 and cases where this treatment was contraindicated are of course not included. Moreover 4 patients who died within the first two days after heparin treatment had finished have also been excluded since reliable prothrombin indices could not be available for the evaluation of the sufficiency of the dosage of anticoagulants. A fifth patient was excluded because death occurred suddenly and no clinical explanation of this could be given autopsy was not permitted. One more case has been excluded as exact information on the initial course was lacking, treatment having been started in one of the surgical wards. Finally one case was excluded because the patient against advice left hospital 12 days after admission and no information as to the course of this case could be had

Results

After these 7 exclusions the series comprises 99 cases. The series has been tatistically evaluated in two different ways

Table I

	Adequately treated	Inadequately treated	Total
0 thrombo-embolism	69	18	87
+ thrombo-embolism	3	9	12
Total	72	27	99

The material has been divided into two groups, the one comprising the cases in which the clinical course and/or the autopsy findings disclosed complications indicating that the anticoagulant therapy had not succeeded the other comprising cases in which no complications of relevance could be ascertained Consideration has been given only to the courses of cases included in this series which as mentioned refer to 1960 The statistical evaluation does not consider the course during earlier or later admissions. The information derived from case records from such admissions is given in the case histories merely to possibly shed light on the course of the case in question. For instance a necropsy finding from a later admission may furnish better understanding of the clinical course and various findings in the treatment during the 1960 admission. Each case-record in both groups was then scrutinized with regard to the prothrombin levels obtained For inadequately treated cases the following definition has been adopted the cases in whom prothrombin indices on more than half the treatment days (reckoned from and including the 3rd day after the last heparin injection) were ≥ 50 whereas cases in whom these values in more than half the treatment days were < 50 have been considered adequately treated This definition is of course arbitrary We have adopted it because we believe it reasonable. Moreover with such a definition the number of patients coming into the two groups becomes large enough to allow statistical treatment. In patients for whom continuous anticoagulant treatment was adopted only the first 35 days of treatment have been considered.

The 99 cases are classified in table I This distribution shows that 79 3 % of the cases *without* thrombo-embolic complications were adequately treated according to the definition given, while only 25 0 % of the cases *with* such complications could be considered adequately treated (fig 1) The difference, 54 3 ± 13 2 % is clearly significant.

The results can also be expressed in the following way in adequately treated cases thrombo-embolic complications occurred in 4.2 % while in inadequately treated cases such complications occurred in 33 3 % (fig 2) The difference here, 29 1 ± 9 4 % is also clearly significant.

The cases in whom anticoagulant therapy did not fulfil its purpose (here tabulated as + thrombo-embolism) number as appears from table I 12 of which 3 were adequately and 9 inadequately treated

The 3 adequately treated were 2 females (67 and 75 years) and 1 male (78 years) the 78-year-old man and the 75-year-old woman had previously had attacks of coronary occlusion. In the male patient autopsy revealed a large cardiac aneurysm, and mural thrombi were found in the aneurysm, moreover a fresh necrosis in the cerebellum was demonstrated. In the femal (75-year-old) autopsy revealed multiple pulmonary infarcts. In the younger female patient ECG, after a week, suggested an extension of the occlusion (right-bundle branch block) the autopsy disclosed widespread myocardial infarction and an adherent mural thrombus in the left ventricle. There

was, furthermore, serous effusion in the pericardium.

The 9 inadequately treated cases include 5 females (57—77 years) and 4 males (45—81 years). In 4 of the females a fresh thrombosis occurred after 6, 5, 5 and 8 weeks respectively. In one of these (72 years) fresh occlusion occurred 1 week after the discontinuation of dicoumarol (the patient survived). In another (77 years) a fresh occlusion occurred while the dicoumarol dosage was being reduced (the patient survived). In a third patient (female, 68 years) treatment had not been started till the third day after admission owing to uncertainty of diagnosis. Six weeks later while still under treatment with dicoumarol, a fresh occlusion occurred and death followed. I fourth patient (70 years) who, 5 weeks after treatment had been started, showed signs of fresh infarction, the autopsy disclosed large, organized infarct in the anterior wall with a fresh marginal infarction. A 5th patient (female, 57 years) had year previously had two attacks of coronary occlusion. She died during this stay in hospital from cerebral embolism (about this case history see below female, 57 years).

In 2 of the 4 males fresh thromboses occurred, in one of them (45 years) after 6 weeks treatment the patient survived. The other patient (61 years) had been discharged after an attack of coronary occlusion and 4 months later (1961) signs of fresh occlusion appeared he was again given anticoagulant therapy. H died and autopsy revealed, besides fibrous and recent myocardial changes, mural thrombi these were considered to stem from the first attack and the corresponding period of treatment. I an elderly male patient (81 years) haematoma at the site of the heparin injections developed 3 or 4 days after admission dicoumarol was discontinued for some days despite the fact that the prothrombin index was 68 the time when the haematoma appeared. A few days later an arterial embolisation in the left lower limb occurred, and the patient weakened and died. Finally there was patient (male 53 years) who developed cerebral thrombosis 3 days after admission he survived.

According to table I there were 87 patients who had no thrombo-embolic complications (0 thrombo-embolism) Nine of these died under treatment. However judging from the clinical course and the necropsy findings the causes of death in these cases were not of a nature which anticoagulant therapy can reasonably be thought to influence. Eight of these were males and 1 was a female (63—83 years). Eight were adequately 1 inadequately treated. Seven of these 9 patients had previously had coronary occlusion the remaining (males, 77 and 63 years) had very extensive infarctions. In one male patient (73 years) necropsy revealed several mural thrombi in the aortic wall. It could not be ascertained whether these had occurred during the period of treatment in 1960 or at an earlier date (for the case history see below male, 73 years).

It has been difficult to assign 2 of our cases to the right category in the table.

Case histories

Female, 57 years. I 1958 admitted for intermittent claudication signs of occlusive vascular disease were found in both lower extremities. In 1959 she was admitted for anterior myocardial infarction and given anticoagulant treatment for 4 weeks a few days after dicoumarol had been discontinued, a recurrent myocardial infarction occurred, this time a posterior wall infarction, and treatment was resumed for 3—4 weeks. During the first period of treatment, the prothrombin index was > 30 for more than half the days of treatment. Now (in 1960) she was admitted with severe precordial pain which did not respond to nitroglycerine. There were only slight ECG changes but pericardial friction sounds were present, GOT values were normal stethoscopical signs of mitral stenosis were found, and ther was period of fibrillo-flutter. Anticoagulant therapy was given. The course was, on the whole satisfactory until 12 day after admission, when signs of cerebral embolism occurred during the next 2 days the patient weakened and died.

Table II

	Adequately treated	Inadequately treated	Total
0 thrombo-embolism	68	19	87
+ thrombo-embolism	4	8	12
Total	72	27	99

Autopsy Old myocardial infarctions were seen in the anterior and the posterior walls and in the septum. An old thrombosis of the anterior descending branch of the left coronary artery Also a stenosis of the mitral valve and rheumatic endocarditis with a few small verrucae on the posterior cusp of the aortic valve were revealed. No mural thrombi Almost the whole left hemisphere of the brain was mollified. At the base of the brain a *complete occlusion of the middle cerebral artery* was found this being due to a firmly lodged *embolic mass* stretching some distance into the artery It cannot be stated whether or not a thrombosis had preceded this embolisation

Prothrombin index On 7 out of 10 days of treatment the prothrombin index was > 50, viz. for 5 days immediately before the cerebral insult, and for 2 days after The treatment must be considered inadequate

COMMENTS

This patient has in table I been placed in the group headed + thrombo-embolism, inadequate treatment This was done because the words embolic mass in the autopsy report were interpreted as an embolus (presumably either from the verrucous endocarditis or from an old mural thrombus) which after the arrival to the brain may have given rise to secondary thrombus formation the detachment of the embolus very probably having been facilitated by the fibrillo-flutter mentioned. If at all a fresh thrombosis, cerebral or mural, did occur during the 1960 period of treatment, the patient indeed belongs to the group in which she has been placed If, on the other hand it is assumed that the embolus stems from a verruca or from an old mural thrombus without subsequent thrombosis, the case should be transferred to the group '0 thrombo-embolism, inadequate treatment because no thrombo-embolic complications could then be stated to have occurred during the 1960 period of treatment.

Male, 73 years. Presumably coronary occlusion in 1956 Angina pectoris for the last 10 years. Now (1960) admitted for posterior myocardial infarction and atrial fibrillation. Anticoagulant treatment was given. The patient was weak during the whole of his stay and died suddenly 4 weeks after admission. No progression in the ECG signs and no clinical signs of embolism.

Autopsy Severe fibrosis in the posterior wall and in the septum and a few fresh, yellow necrotic areas were seen. Severe coronary arteriosclerosis, the right branch being occluded for the first 3—4 cm, the left artery stenosed In the circumflex branch of the left coronary artery a haemorrhage in the wall and a considerable (old as well as fresh) thrombotic mass in the lumen. No mural thrombi in the heart were found but *extensive thrombi in the aortic wall.* It cannot be stated whether these date from the period of treatment.

Prothrombin index On only 3 of 23 days of treatment was the prothrombin index > 50. The treatment was, thus, adequate

COMMENTS

In this case it is open to discussion whether the mural thrombi found in the aortic wall are of old or recent origin. The case has been placed in table I in the group headed 0 thrombo-embolism, adequate treatment on the probability that the thrombi were of old date. If the thrombi have been formed during the actual period of illness (i e. in 1960) the purpose of the anticoagulant treatment has not been achieved and the case should

be transferred to the group + thrombo-embolism, adequate treatment which comprises 3 cases.

If both cases are transferred the distribution will be as in table II

It appears from this table that 78.2 % of the cases without thrombo-embolic complications were adequately treated as against 33.3 % of the cases *with* such complications. The difference is 44.9 ± ± 14.3 %. Expressed otherwise among adequately treated cases, 5.6 % had thrombo-embolic complications, and among inadequately treated 29.6 %. The difference here is 24.0 ± 9.2 %. Hence the differences are still significant no matter whether starting from the total number of adequately treated, the cases are grouped into cases with and cases without thrombo-embolic complications or whether starting from the number of cases with thrombo-embolic complications, the series is grouped into cases adequately treated and cases inadequately treated.

For the sake of completeness it should be mentioned that altogether 17 of the 99 patients died, 8 with thrombo-embolic complications (3 adequately, 5 inadequately treated) and 9 without thrombo-embolism (8 adequately, 1 inadequately treated)

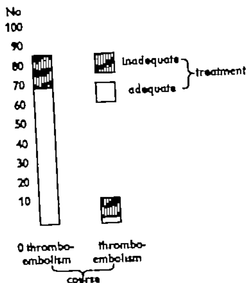


Fig. 1 Distribution of adequately and inadequately treated cases among patients with and without thrombo-embolic complications respectively during the period of treatment.

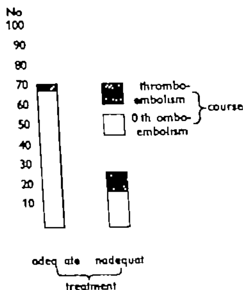


Fig. 2. Distribution of cases with and without thrombo-embolic complications during the period of treatment among the adequately and the inadequately treated patients respectively

Discussion and conclusion

A series consisting of 99 consecutive cases of acute myocardial infarction treated with anticoagulants was analysed to ascertain if there was any correlation between the efficiency of the anticoagulant therapy and the occurrence of thrombo-embolic complications. Daily prothrombin determinations (Quick-method) were carried out. A prolongation of the prothrombin-time t twice the normal value was

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seems to demonstrate that anticoagulant therapy is indicated in acute myocardial infarction. The importance of keeping the prothrombin index within the therapeutic range for a sufficiently long time is stressed, likewise the advisability of a gradual decrease of dicoumarol dosage in preference to abrupt discontinuation.

Acknowledgement

I wish to express my thanks to Dr Willy Munck, Professor of Pathology University of Aarhus, for his kind assistance in explaining some dubious points in the autopsy findings.

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considered sufficient if this reduction of the prothrombin content was achieved on at least 50 % of the treatment days. These cases were defined as adequately treated cases whereas cases in whom such a reduction of prothrombin for at least 50 % of the treatment days was *not* achieved have been termed inadequately treated

The incidences of thrombo-embolism (clinically and in necropsy) in the two groups were significantly different in the adequately treated cases thrombo-embolic complications were very infrequent in sharp contrast to the incidence in inadequately treated

In one case with inadequate treatment there has been some doubt as to whether thrombosis has occurred in connection with cerebral embolism In another case where the treatment had been adequate it was uncertain whether some parietal thrombi in the aorta had been formed while the anticoagulant therapy had been in progress or whether they were of an older date In the statistical calculations these two cases have been placed in both possible categories with no detriment to the significance of the difference found

In this investigation no attempt has been made to evaluate the series according to the fatal and non fatal outcome since its prime object has been to assess the frequency of those complications of myocardial infarction which anticoagulant therapy according to our present knowledge can be presumed to prevent or restrict — and thereafter to evaluate the relationship between these and the efficiency of the treatment. However no reasonable doubt can exist that the occurrence of such complications will induce an increased mortality Furthermore in view of the present object it is of little interest to investigate to what extent the patients have died from causes which cannot be thought to be influenced by the treatment.

In conclusion it must be said that if the treatment is to achieve its purpose, it is of utmost importance that the level of the prothrombin index of the plasma should be brought down to what is considered the therapeutic range. Bleeding during treatment occurred in two cases only and as special circumstances were present in both these cases, our observations seem to indicate that a somewhat bolder dosage might produce better results. The course of the disease in some of the cases seems to call for caution in discontinuation of the treatment a gradual decreasing is to be preferred to a sudden discontinuation and in all severe cases anticoagulants should not be reduced until the patient appears to be well within the safety margin, and not until at least five weeks after the occurrence of the infarction in such cases reduction should be done very gradually

Finally the results strongly suggest that anticoagulant therapy is indicated for patients with acute myocardial infarction, provided that the principles laid down here are followed

Summary

Ninety nine patients treated with anticoagulant therapy including 97 cases of acute myocardial infarction have been evaluated with regard to the relation between the intensity of treatment (as measured by the level of prothrombin achieved) and the incidence of complications of the type assumed to be influenced by this therapy Among inadequately treated patients such complications occurred with a significantly higher incidence than among adequately treated cases. This

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Effect of Deoxyuridine on Incorporation of Tritiated Thymidine: Difference Between Normoblasts and Megaloblasts

By

SVEN-AAGE KILLMANN

The two most common causes of megaloblastic anemia are vitamin B_{12} and folic acid deficiency. It is firmly established that derivatives of folic acid are involved in the biosynthesis of purines and thymine and hence of deoxyribonucleic acid (DNA) (2, 21) whereas the possible role of vitamin B_{12} in the synthesis of DNA and its precursors is controversial (24). Clinically it is well known that folic acid can induce long-standing remissions in megaloblastic anemia due to vitamin B_{12} deficiency (27). This suggests some common site(s) of action of the two vitamins, or perhaps rather a combined action of the vitamins since the remissions of pernicious anemia from folic acid are of limited duration.

A key reaction in pyrimidine biosynthesis is the methylation of deoxyuridylic acid to thymidylic acid. This process requires tetrahydrofolic acid (11, 19). The results of the present study suggest that this reaction also depends on vitamin B_{12}.

Methods

1. Thymine-free deoxyuridine (Calbiochem) was dissolved in 0.9% saline (1 μM/ml) and 0.1 ml of this solution (= 0.1 μM deoxyuridine) placed in a small test tube. A control tube received 0.1 ml of saline.

2. Bone marrow was aspirated from the iliac bone into a syringe containing a few drops of saline with heparin (heparin Leo® 1 mg/ml saline).

In order to break up the marrow particles, the contents of the syringe were repeatedly passed through a fine needle. Equal volumes of bone marrow (0.4–1.7 ml, depending on the yield of the aspiration) were then immediately transferred to the saline and deoxyuridine tube by means of a calibrated tuberculin syringe.

3. After thorough mixing, the tubes were left at room temperature for 1 hour with agitation at intervals. Then 0.2 ml of saline containing 2 μC of tritiated thymidine (1.9 C/mM, Schwarz Bio Research, Inc., Orangeburg, N. Y.) was added to each tube.

4. After 1 hour's incubation with tritiated thymidine at room temperature the reaction was stopped in an ice bath. Following brief centrifugation smears were prepared from the sediment, immediately dried in a stream of cold air and fixed in absolute methanol.

5. Autoradiograms were prepared with Kodak AR 10 stripping film or Kodak NTB-2 emulsion (diluted 1:1 with distilled water). The slides were exposed at 4° C. Exposure time was chosen in such a way that a distinct difference between truly labelled cells and cells "labelled" due to background was obtained. In most experiments this was achieved

Submitted for publication September 30, 1963.

Table I. Bone marrow cells were incubated with saline (control) or deoxyuridine for 1 hour and labelled with H^3-thymidine. The table shows the effect of preincubation with deoxyuridine on incorporation of H^3 thymidine in megaloblasts of untreated pernicious anemia (p.a., patients 1a—7) and in normoblasts (patient 1b and 8—12). One patient with pernicious anemia was studied before (1a) and after treatment with vitamin B_{12} (1b). The change in labelling resulting from preincubation with deoxyuridine was computed from the mean grain counts (see text).

Pat. no.	Diagnosis	After incubation with saline			After incubation with deoxyuridine			% change with deoxy-uridine
		No. of mitotable cells counted	% labelled	Total grains	No. of mitotable cells counted	% labelled	Total grains	
1	p.a.	97	49.5	836	91	49.5	768	− 4.5
2	p.a.	68	34.9	315	52	46	312	+25
3	p.a.	93	33.3	632	90	36.6	747	+22
4	p.a.	178	43.7	1,296	92	44.5	615	− 6
5	p.a.	96	54.2	1,218	100	54	974	−23
6	p.a.	84	40.5	318	87	30	186	−43.5
7	p.a.	91	56.3	928	90	14.5	216	−76.5
1 b	p.a. after B_{12}	108	31.3	470	123	0	0	−100
8	Osteoporosis	137	32.0	557	201	1.5	20	−97.5
9	Liver cirrhosis	96	51.0	795	96	3.2	34	−95.7
10	Iron def. anemia	103	85.0	1,277	104	12.5	117	−91.5
11	Iron def. anemia	98	45.0	863	134	13.4	194	−85.3
12	Refractory anemia	50	46.0	416	47	19.1	82	−79.1

Folic acid, on the other hand, is known to be involved in the synthesis of DNA precursors, i.e. a tetrahydrofolic acid derivative, 5,10-methylenetetrahydrofolate, is required for the biosynthesis of thymidylic acid (32). Clinically it is well established that folic acid can induce long lasting remissions of vitamin B_{12}-deficiency megaloblastic anemia ([illegible]). Therefore, it seemed desirable to investigate whether vitamin B_{12} is connected with the synthesis of the thymine moiety of DNA in human cells.

Briefly, the *de novo* synthesis of thymidylic acid involves the following steps: low molecular compounds → orotidylic acid → uridylic acid → deoxyuridylic acid → thymidylic acid. The latter reaction is a methylation of the uracil base and requires tetrahydrofolic acid (11, 19, 32). These conversions take place at the nucleotide level; thus thymidine (that is, thymine deoxyriboside, a nucleoside) is not a normal precursor of DNA. However, exogenously supplied thymidine is readily and specifically utilized for DNA synthesis *in vitro* and *in vivo* (21, 25). In the cell thymidine is phosphorylated to thymidine monophosphate (thymidylic acid) and thymidine triphosphate and then incorporated into DNA (4, 20). Similarly, deoxyuridine (that is, uracil deoxyriboside) is not a normal DNA precursor but when supplied it is utilized as a precursor of the thymine moiety of DNA after phosphorylation to deoxyuri-

by 7 days exposure but occasionally longer exposure was necessary After development (Kodak D-19 b developer 6 min for AR 10 2 min. for NTB-2, Kodak X ray fixer 6 min. for AR 10 2 min. for NTB-2) the slides were stained with Giemsa at pH 5 75

6 Only slides which had been exposed developed, and stained together were compared. At least two slides from each tube were counted. Fifty or one hundred red cell precursors were counted in each slide The smallest and largest nuclear diameter of each cell was measured, and the number of grains over ying the nucleus recorded Cells with five grains or more were classified as labelled. Nuclear size measurements were considered essential since the intensity of labelling varies with the nuclear size of red cell precursors, the smallest (most mature) red cell precursors do not incorporate thymidine at all. From the control tube data it was determined at which nuclear size level labelled cells occur red. Cells with this nuclear size or larger will be referred to as mitotable From analysis of the nuclear size data it was ascertained that the counted samples of mitotable cells from the control and deoxyuridine tubes were comparable with respect to size distribution.

Results

The results are presented in table I which indicates the number of mitotable red cell precursors counted in each marrow sample, the percentage of labelled mitotable erythroblasts (i. e. cells with five grains or more) and the total number of grains counted in the labelled cells. The mean grain count of all (labelled and unlabelled) mitotable cells was computed by dividing the total number of grains in the sample by the total number of mitotable cells. The last column to the right shows the difference between the mean grain counts of the saline controls (taken as 100 %) and the deoxyuridine treated cells.

In normoblasts, incubation with deoxyuridine prior to H^3-thymidine labelling caused an 80—100 % decrease in H thymidine incorporation. In contrast, H^3 thymidine incorporation into megaloblasts was unaffected by deoxyuridine in five cases, moderately reduced in one case, *and only in one pernicious anemia mar*row did the reduction in H^3 thymidine uptake approach that observed in normoblasts.

Discussion

The biochemical role of vitamin B_{12} is far from clear As a growth factor for certain bacteria it can be replaced by a number of pyrimidine deoxyribosides (17) This led to the hypothesis that vitamin B_{12} is involved in the biosynthesis of deoxyribose. Subsequently more direct evidence for this has been obtained in bacteria (7 28) and murine cells (18) However other studies in bacteria (29) fowl (3) and pigs (31) do not support the concept that deoxyribose synthesis depends on vitamin B_{12}. The subject has been reviewed recently (1 2)

Because of the profound effect of vitamin B_{12} on blood cell formation in man it has been suspected that the vitamin is connected with DNA-synthesis. Studies in bacteria (23 30) and human megaloblasts in vitro (26) suggest that overall DNA-synthesis is enhanced by vitamin B_{1} Evidence has been presented that vitamin B is connected with the synthesis of thymine methyl in bacteria (5) and chick bone marrow (3 6) However results to the contrary have been reported as well (31) and lately doubt has been expressed with regard to the possible role of vitamin B_{1} in DNA-synthesis (24) As appropriately pointed out by Bolinder and Reichard (3) the varying results might be due to variations in the relative sensitivities of several B_{1}-dependent reactions in different organisms.

de novo synthesis of DNA-thymine is impaired in vitamin B_{12}-deficiency. This would lead to a state of relative thymine starvation. Circumvention of the supposed metabolic block by administration of preformed thymidine to patients with pernicious anemia would then be expected to produce a hematopoietic response. Recent work shows that in untreated pernicious anemia, thymidine will produce reticulocytosis, decrease in serum iron concentration, rise in leukocyte and platelet count, and partial conversion of megaloblastic to normoblastic erythropoiesis (14). These results strongly suggest that thymine deficiency is an important aspect of vitamin B_{12}-deficiency and indirectly support mechanism 5.

This conclusion is in discord with the results of Lessner and Friedkin (17 a) who found no impairment of C^{14}-deoxyuridine incorporation into DNA of megaloblastic bone marrow. Comparisons with the present study are difficult, however, because of differences in experimental design. The approach of Lessner and Friedkin is more direct than that of this study but involves more manipulation of the cells and refers to all bone marrow cells as a whole. Also since they determined the specific activity of DNA (not DNA thymine) it is possible, although perhaps not likely, that their results could in part be explained from conversion of C^{14}-deoxyuridine to C^{14}-deoxycytidylic acid with subsequent incorporation into DNA. Studies on the incorporation of labelled deoxyuridine into DNA-thymine of megaloblasts and normoblasts are clearly needed.

In some megaloblastic marrows of the present study (table I) deoxyuridine did not reduce H^3-thymidine incorporation at all. Obviously these results pertain only to the particular conditions of the experiment and do not necessarily indicate a complete block of thymidylic acid synthesis. Indeed, it is known from direct analysis that DNA of pernicious anemia bone marrow does contain thymine (8).

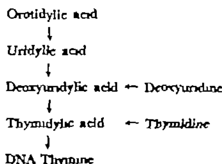
It is unlikely that the suggested effect of vitamin B_{12} on the conversion of deoxyuridylic to thymidylic acid is a direct one. In cell free systems no evidence has been found that vitamin B_{12}-coenzymes participate directly in thymidylate synthesis (9, 32). More likely the effect of vitamin B_{12} is one on folic acid coenzymes. Although some studies have failed to disclose aberrations in the metabolic functions of folic acid in pernicious anemia (10), other reports (reviewed in ref. 13 and 27) provide increasing evidence that folic acid metabolism is deranged in vitamin B_{12}-deficiency: formiminoglutamic acid excretion is frequently increased (12, 16), folic acid plasma clearance is more rapid than normal (13), and in many pernicious anemia patients there is "pile up" of *L. casei*-active serum folate activity which disappears abruptly after cyanocobalamin therapy (13).

The approach of the present study should be applicable to related studies of factors affecting DNA-synthesis in blood and bone marrow cells. The main disadvantage of the procedure is the limited accuracy of quantitative microautoradiography which may render detection of subtle effects difficult. Obvious advantages are that studies can be performed with small amounts of cellular material and that it can be ascertained that the cells which are studied and compared are cytologically homogenous.

Conclusion and summary

Bone marrow from patients with untreated pernicious anemia and from pa-

Table II Synthetic pathway of thymine moiety of DNA Deoxyuridine and thymidine are readily utilized for synthesis of DNA thymine but are not normal DNA-precursors



dylic acid which in turn is methylated to thymidylic acid (21) The reactions are summarized in table II

Incubation of human leukocytes with excess of non radioactive deoxyuridine will reduce the uptake of subsequently added H^3-thymidine (15) This indicates that the uptake of deoxyuridine by the cells and its conversion to thymidylic acid is efficient enough to allow it to compete with thymidine in the reactions leading to DNA thymine. Impairment of the methylation of deoxyuridylic to thymidylic acid would be expected to reduce the competitive effect of deoxyuridine since there is no alternative pathway by which it could enter DNA thymine Therefore, if the conversion of deoxyuridylic acid to *thymidylic acid* is vitamin B_{12} dependent deoxyuridine should inhibit H thymidine incorporation less in vitamin B_{12}-deficient cells than in normal cells

In the present experiments, the amount of deoxyuridine added to each tube was 0 1 μM. The amount of chemical thymidine added with 2 μC of H^3 thymidine (specific activity 1 9 C/mM) was about 0 001 μM In normoblasts preincubation *with deoxyuridine* reduced H^3 thymidine labelling by 80—100 % In megaloblasts, deoxyuridine had much less or no effect at all on the incorporation of H^3 thymidine (table I).

In an attempt to explain this difference between normoblasts and megaloblasts, several possibilities must be considered

1 Deoxyuridine will pass the cell membrane of normoblasts but not of megaloblasts whereas thymidine can penetrate freely into both cell types.

2 In normoblasts, but not in megaloblasts deoxyuridine will induce an enzyme which splits not only deoxyuridine but also thymidine. Inducible enzymes of this type have been found in several cell systems (22) Enzyme would be formed during preincubation with deoxyuridine, and thymidine which is added later would be rapidly degraded to thymine which is a poor DNA precursor

3 Deoxyuridine is phosphorylated in normoblasts but not in megaloblasts whereas thymidine is phosphorylated in both cell types.

4 Enzyme saturation from deoxyuridine takes place more readily in normoblasts than in megaloblasts.

5 Deoxyuridylic acid is methylated to thymidylic acid in normoblasts but not in megaloblasts.

From the present data it cannot be decided which of these possible mechanisms is operative However mechanism 5 is the most attractive one considering that the conversion of deoxyuridylic to thymidylic acid requires tetrahydrofolic acid, and folic acid deficiency causes megaloblastic anemia just as vitamin B_{12} deficiency does Mechanisms 1—4 would be inconsequential to the *de novo* synthesis of DNA thymine *in vivo* (cf table II) although mechanism 4 would be compatible with a salvage pathway for DNA synthesis in pernicious anemia. In contrast, mechanism 5 would imply *that the*

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Erythropoietic Response to Thymidine in Pernicious Anemia

By

Sven-Aage Killmann

The present study was prompted by recent observations on the in vitro incorporation of H^3-thymidine in human erythroblasts (12). In normoblasts, H^3 thymidine incorporation was almost completely inhibited by prior incubation with deoxyuridine. In contrast, deoxyuridine caused little or no reduction of H thymidine uptake in megaloblasts of pernicious anemia.

These data suggested that the conversion of deoxyuridylic acid to thymidylic acid is impaired in vitamin B_{12} deficiency. If this supposition is correct, *circumvention of the presumed metabolic* block by administration of preformed thymidine to patients with pernicious anemia would be expected to produce a hemopoietic response.

Material and methods

One patient with acute erythroleukemia (di Guglielmo) and fiv patients with pernicious anemia were studied. The diagnosis of pernicious anemia was based on the following criteria: (1) macrocytic anemia (2) megaloblastic bone marrow (3) subnormal vitamin B_{12} concentration (4) achlorhydria after histamine test meal, and (5) reticulocyte response and rise in hemoglobin concentration after treatment with vitamin B_{12}.

Serum folic cid activity (L. leichmannii assay (14, 15)) was determined in all patients but one (E. B.) and was within the normal range.

All patients were on regular hospital diet, *and none of them received corticosteroids*, antibiotics or hematinics prior to or during the period of study.

Thymidine (A grade, California Corporation for Biochemical Research) was dissolved in redistilled water (50 mg/ml, later stock solution of 25 mg/ml was found more satisfactory since with the higher concentration crystallization is likely to occur on prolonged standing at 4 C). The material was sterilized by Seitz filtration and tested for pyrogens. Material from tw production batches has been used and found effective.

The dose of thymidine was estimated very roughly as follows: deoxyribonucleic acid (DNA)-content of single cell 6×10^{-12} g (17). With equimolar amounts of nucleotides, the content of thymidine per cell will be bout 10^{-13} g. The bone marrow of normal man contains bout $3—4 \times 10^{12}$ mitotable cells (13). I pernicious anemia, this figure will be higher say 10^{13} cells. Other rapidly proli-

Submitted for publication September 30, 1963.

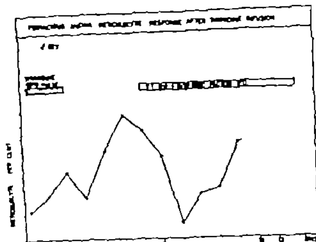
tients with normoblastic erythropoiesis were incubated with deoxyuridine and subsequently labelled with H^3 thymidine. The uptake of H^3 thymidine in erythroid precursors was studied microautoradiographically. In normoblasts deoxyuridine caused an 80—100 per cent decrease in H^3 thymidine incorporation. In contrast deoxyuridine had little or no effect on H^3 thymidine uptake in megaloblasts. These data combined with observations on the hemopoietic effect of thymidine in untreated pernicious anemia strongly suggest that vitamin B_{12} is required for the methylation of deoxyuridylic acid to thymidylic acid.

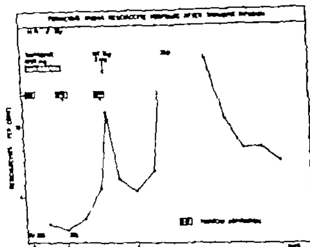
Acknowledgements

The author is highly indebted to Dr. Jørgen Jørgensen, Sygekasselægernes Organisations Labor., Copenhagen, for his help in referring patients. This investigation was supported by PHS Research Grant CA 5492 HEM from the National Cancer Institute, U. S. Public Health Service.

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Fig. 3. J. M. Pernicious anemia. Hemoglobin concentration 8.9 g/100 ml. Thymidine infusion was maintained for 48 hours. Dose of vitamin B_{12} orally 45 μg daily


Fig. 4. M. N. Pernicious anemia. Hemoglobin concentration 6.8 g/100 ml. Thymidine infusion was maintained for 48 hours. F = serum iron concentration (μg/100 ml)

As shown in figs. 1—5 a definite reticulocytosis occurred in all five pernicious anemia patients following thymidine. In spite of the reticulocyte response, no rise in hemoglobin concentration was observed in patient K. L. The remaining patients were not followed long enough to decide whether the hemoglobin concentration increased after thymidine.

Leukocyte and platelet counts were followed only in patient K. L. A distinct but temporary rise in both counts took place after thymidine (fig. 5).

The serum iron concentration was studied in three patients. The data are included in figs. 1, 4 and 5. No change was found in M. N. (fig. 4) after 48 hours thymidine infusion. In E. B. (fig. 1) a moderate decrease was seen 48 hours after thymidine had been started, and in K. L. (fig. 5) there was a marked decrease after 72 hours treatment, followed by a rise when the infusion had been completed. Simultaneous transferrin determinations in all three patients showed only minor fluctuations.

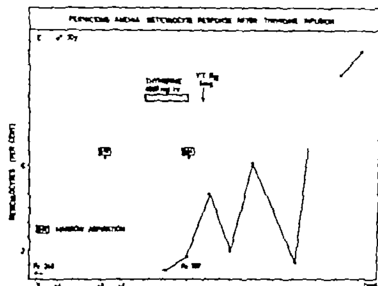


Fig. 1. E. B. Pernicious anemia. Hemoglobin concentration 6.6 g/100 ml. Thymidine infusion was maintained for 48 hours. Fe = serum iron concentration (μg/100 ml)

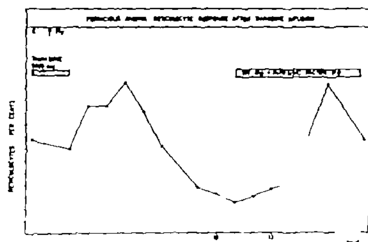


Fig. 2. E. L. Pernicious anemia. Hemoglobin concentration 6.8/100 ml. Thymidine infusion was maintained for 48 hours. Dose of vitamin B_{12} orally 45 μg daily

ferating tissues are the gut, skin, and lymphoid tissues. It was assumed that the total number of proliferating cells in the body was in the order of 2×10^{12}. With a roughly estimated average generation time of 24 hours, $2 \times 10^{12} \times 10^{-12}$ g = 2 g of thymidine is needed for DNA-synthesis per day if all thymidine must be supplied from exogenous sources. This is not the case, however since thymidine is known to be present in DNA of pernicious anemia marrow (8). On the other hand, an unknown but probably large fraction of exogenous thymidine will be catabolized by the liver. During the study it was found that thymidine is readily catabolized also in pernicious anemia: studies of the urinary excretion of β-aminoisobutyric acid, a specific catabolite of thymidine, showed a marked increase in the output of this substance during the infusion of thymidine.

Prior to use 2.0–3.0 g of thymidine was added to 1 000 ml of 5% glucose. This solution was given to the patients as a continuous infusion at a constant rate. The infusion was maintained for 48–72 hours. The total dose received by each patient is indicated in fig. 1–6. Reticulocytes were counted per 1 000 red cells in wet preparations.

Results

Thymidine infusion was well tolerated by all patients except for occasional mild superficial phlebitis of the infusion vein

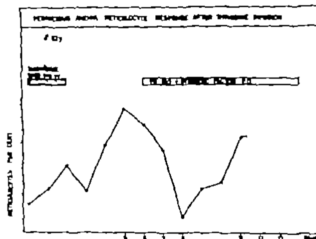


Fig. 3. J. M. Pernicious anemia. Hemoglobin concentration 8.9 g/100 ml. Thymidine infusion was maintained for 48 hours. Dose of vitamin B_{12} orally 45 µg daily

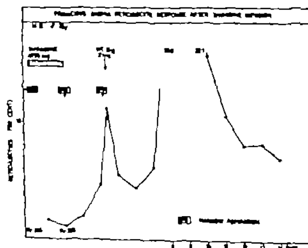


Fig. 4. M. N. Pernicious anemia. Hemoglobin concentration 6.8 g/100 ml. Thymidine infusion was maintained for 48 hours. F = serum iron concentration (µg/100 ml)

As shown in figs. 1—5 a definite reticulocytosis occurred in all five pernicious anemia patients following thymidine. In spite of the reticulocyte response, no rise in hemoglobin concentration was observed in patient K. L. The remaining patients were not followed long enough to decide whether the hemoglobin concentration increased after thymidine.

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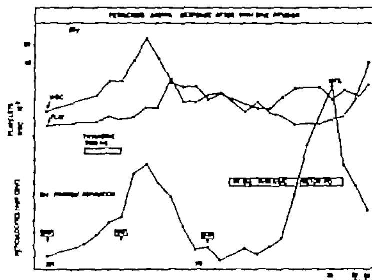


Fig 5 K. L. Pernicious anemia. Hemoglobin concentration 7 0 g/100 ml. Thymidine infusion was maintained for 72 hours. Dose of vitamin B_{12} orally 45 μg daily Fe = serum iron concentration (μg/100 ml)

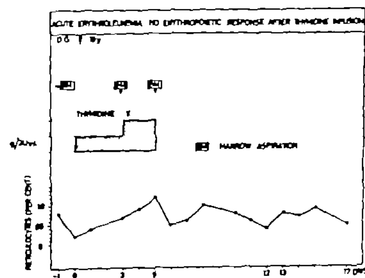


Fig 6. Acute erythroleukemia (di Guglielmo) Hemoglobin concentration 6.7 g/100 ml Thymidine infusion was maintained for 190 hours without effect on reticulocyte count (note scale) The bone marrow remained megaloblastic.

Bone marrow studies after thymidine were done in three patients. The time of aspiration is shown in figs. 1 4 and 5 In E. B megaloblastosis persisted after thymidine technical imperfections prevented detailed studies. In M N and K. L. definite changes had taken place. Although the marrow still contained many typical megaloblasts, there was a considerable increase in the proportion of "intermediate erythroblasts ("intermediate megaloblasts of Dacie and White (3)) and normoblasts. In table I a quantitative expression of the changes has been attempted The difficulties inherent in this type of cytological classification are clearly recognized, and great care was taken to maintain as constant criteria for cell classification as possible. Thymidine infusion was followed by a definite decrease in immature megaloblasts ("basophilic megaloblasts" in table I comprising promegaloblasts and smaller basophilic megaloblasts) and in the characteristic large polychromatic megaloblasts which have a cell size 3—4 times

Table I Differential counts of red cell precursors in the bone marrow of two patients treated with thymidine (M. N. 48-hour infusion of 4.75 g; K. L. 72-hour infusion of 9 g). Two or three hundred red cell precursors were counted at each time interval. The term "intermediate erythroblasts" was used for the cell types called "intermediate megaloblasts" by Dacie & White (3). The frequency of the various cell types is given as percentage of all nucleated red cell precursors

	M. N.			K. L.		
	Before thymidine	Immediately after thymidine	2 days after completion of thymidine	Before thymidine	Immediately after thymidine	7 days after completion of thymidine
Megaloblasts						
Basophilic	15.0	5.0	20.5	24.7	10.3	18.3
Polychrom. large	28.0	11.0	17.5	14.7	5.7	13.7
Polychrom. small	23.0	19.0	23.5	28.3	12.0	37.3
Orthochrom.	10.5	12.5	14.5	13.0	11.0	6.3
Intermediate erythroblasts						
Basophilic	6.5	8.0	4.5	5.0	11.0	5.0
Polychrom.	4.0	15.5	3.5	4.0	10.7	6.3
Orthochrom.	2.0	8.0	5.5	2.0	4.0	1.0
Normoblasts						
Basophilic	1.0	3.5	1.5	0.3	8.0	2.7
Polychrom.	2.5	13.5	2.0	2.7	15.0	4.3
Orthochrom.	7.5	6.0	8.0	5.3	14.3	5.0

that of a red cell. Instead there was a marked rise in polychromatic intermediate erythroblasts and polychromatic normoblasts. After thymidine had been discontinued the marrow rapidly reverted to its former appearance.

The patient with erythroleukemia had previously been treated with 6-mercaptopurine but medication had been stopped two weeks prior to the study. Megaloblasts were abundant in the marrow. Thymidine had no effect on the marrow nor on the reticulocyte count (fig. 6).

Discussion

Various pyrimidine precursors have been studied in the past with respect to their clinical effect in pernicious anemia. Slight reticulocyte responses have been observed from ureidosuccinic acid given orally (20). Treatment with orotic acid, 3—6 g daily by mouth, has produced sustained remissions in some but not all patients. In spite of definite reticulocytosis and rise in hemoglobin, the bone marrow remains megaloblastic (20). Massive oral doses of uracil resulted in remission and restoration of normoblastic erythropoiesis in one of three patients (25). Large doses of thymin (6 g or more daily) by mouth resulted in reticulocytosis, rise in hemoglobin and conversion of megaloblastic to normoblastic erythropoiesis (22, 25). The response is dose dependent (22). Effect has been noted also in a pernicious anemia patient with secondary resistance to folic acid (25).

The results obtained with thymidine are contradictary Hausmann (9) observed reticulocyte crisis rise in hemoglobin and restoration of normoblastic erythropoiesis in two patients after 200—280 mg of thymidine intramuscularly daily for ten days. Reisner and West (18) observed slight reticulocytosis after single injections of small doses (5—150 mg) of thymidine. On the other hand Ungley (24) observed no effect from a small dose (48 mg) of thymidine i m in one subject. Spray and Witts (23) treated five pernicious anemia patients with 250—500 mg of chromatographically pure thymidine i.m. daily for 8—14 days A moderate reticulocyte response and rise in hemoglobin took place in only one patient. Similarly Vilter et al (26) did not observe remissions with thymidine. In megaloblastic anemia of tropical sprue, thymidine (i m up to 250 mg daily for 10 days) had no effect whatever (2)

Thus, most attempts to obtain a hemopoietic response in pernicious anemia with thymidine have been unsuccessful A notable exception is the report by Hausmann (9) However his thymidine preparation was made from purified liver extract. One may suspect that the preparation was contaminated with vitamin B_{12} or folic acid although tests for these compounds were reported to be negative In any event no effect has been observed with chromatographically pure thymidine, administered in similar doses and for the same length of time (23)

In the present series thymidine was followed by a definite reticulocyte response in all patients with pernicious anemia. In contrast the patient with di Guglielmo's syndrome (fig 6) did not respond although the marrow contained large numbers of megaloblasts. This attests to the specificity of the effect in the vitamin B_{12}-deficient patients. Apart from reticulocytosis, thymidine also resulted in rising leukocyte and platelet counts and in a decrease of the serum iron concentration in two of three patients. Finally megaloblastic erythropoiesis was in part converted to normoblastic erythropoiesis.

The discrepancy between the present data and the results of Spray and Witts (23) and others (18 24) may be explained from differences in dose and/or administration of thymidine. The daily doses used in this study were 5—10 times larger than those given by Spray and Witts The estimate of the required thymidine dose is so uncertain that it is not possible to decide whether this difference is important although comparisons of the reticulocyte responses produced by thymidine and subsequent vitamin B_{12} therapy may suggest that even the dosage employed in this study was suboptimal (figs 1 4 5) An important point may be that in the present series thymidine was administered as a constant intravenous infusion rather than as intramuscular injections (2 23) Recent studies employing tritium labelled thymidine have shown that thymidine is extremely rapidly cleared from the plasma and either incorporated into DNA or rapidly catabolised (19)

It is unlikely therefore that injections once or twice daily will provide a continuous supply of thymidine If thymidine could be stored in cells prior to DNA synthesis, intramuscular administration might still be adequate. However there is good evidence that thymidine is incorporated into cells only during DNA synthesis (6) and moreover the rapid attainment of maximal cell labelling after injections of H thymidine in vivo (19) would be inconsistent with any

significant storage of thymidine prior to DNA-synthesis. Of mitotable megaloblasts, about 35—55 per cent are in DNA-synthesis at any one time (12). The duration of DNA-synthesis in megaloblasts is not known but from data on other mammalian cells (11) it is likely to be several hours. Probably previous failures to demonstrate a hemopoietic effect of thymidine in vitamin B_{12} deficiency anemia have in large measure been due to discontinuous availability of thymidine to marrow cells resulting from the intermittent administration of the material. The relative importance of dose and mode of administration can be approached experimentally.

As pointed out by Spray and Witts (23) it appeared enigmatic why thymine would and thymidine would not induce remissions in pernicious anemia, in particular since thymine is a poorly utilized precursor of DNA whereas thymidine is readily phosphorylated to thymidylic acid and incorporated into DNA (16, 17). In part, this problem is resolved with the present demonstration of the effectiveness of thymidine. Mass action may be an important factor in the results achieved with thymine. It should be noted that thymine has been used (22, 25) in comparatively much larger doses (6—15 g) than thymidine, albeit by mouth. However, studies on the urinary excretion of β-aminoisobutyric acid (BAIBA), a specific catabolite of thymine, indicate that thymine is rapidly and efficiently absorbed from the gut (1, 7). The fate of that fraction of absorbed thymine which does not go down the reductive pathway to BAIBA is not known. In part, it might be converted to thymidine by transdeoxyribosidation in the liver (or bone marrow). A rat liver deoxyribonucleoside-transferring enzyme has recently been described by de Verdier and V. R. Potter (5).

The reported effects of carbamylaspartic acid, orotic acid and uracil (20, 25) in pernicious anemia are more difficult to understand, in particular if it is correct that methylation of deoxyuridylic acid to thymidylic acid is impaired in vitamin B_{12} deficiency. Again, mass action may be important since the presumed block in methylation is not complete (12). Spontaneous remission in some of the reported cases is another possibility.

The requirement of tetrahydrofolic acid for the methylation step in thymidylic acid synthesis is well established (4). The possible role of vitamin B_{12} in nucleic acid synthesis is less clear (4, 12, 21). Recent observations on human pernicious anemia megaloblasts in vitro suggested an impairment of the methylation of deoxyuridylic acid to thymidylic acid in vitamin B_{12} deficiency (12). If so, the bone marrow and other tissues in pernicious anemia would seem to be in a state of relative thymine starvation, and administration of the thymine moiety in a form which can be utilized for DNA synthesis would be expected to produce a hemopoietic response. The present results strongly suggest that vitamin B_{12}-deficient man is indeed relatively thymine-deficient, and thus indirectly support the hypothesis that vitamin B_{12} is involved in the methylation of deoxyuridylic acid to thymidylic acid.

Qualitatively, the changes induced by thymidine are similar to the initial effect of therapy with vitamin B_{12} or folic acid. Quantitatively, thymidine was less effective than vitamin B_{12}; reticulocytosis was slighter and the marrow did not become completely normoblastic. It remains to be seen whether this was due to insufficient dosage of thymidine.

The partial conversion to normoblastic erythropoiesis induced by thymidine could be explained in two ways single or combined 1) Exogenous thymidine has a sparing action on endogenous thymidylate synthesis hence more tetrahydrofolic acid and/or vitamin B_{12} will be made available for other synthetic reactions, hitherto impaired which are needed for normoblastic, as opposed to megaloblastic cell development. 2) Megaloblastic cell changes are a direct result of thymine deficiency and therefore thymidine causes them to regress. A consequence of the first possibility would be that other substances requiring vitamin B_{12} and tetrahydrofolic acid for their synthesis *e g* methionine, should induce a hemopoietic response in pernicious anemia. There is good evidence that methionine does influence folic acid metabolism in vitamin B_{12}-deficient animals (reviewed in ref. 10) Although a good response to combined methionine and choline has been observed in a patient with liver cirrhosis and folic acid deficiency (25) methionine alone has had no effect in pernicious anemia (20) It seems more likely therefore, that megaloblastosis will turn out to be a direct result of impaired thymine synthesis but definite conclusions are not warranted at the present time

Summary and conclusions

Five patients with pernicious anemia treated with continuous i v infusion of thymidine all responded with reticulocytosis. Other effects were a decrease in serum iron concentration and an increase in leukocyte and platelet counts. The bone marrow showed definite changes with increasing numbers of normoblasts and intermediate erythroblasts The data strongly suggest that thymine deficiency is an important aspect of vitamin B_{12} deficiency in man It is possible but not proven that the megaloblastic cell changes are caused at least in part, by impaired biosynthesis of the thymine moiety of DNA.

No response to thymidine was seen in a patient with megaloblastic anemia in the course of acute erythroleukemia.

Acknowledgement

The author is highly indebted to Dr Jorgen Jorgensen, Sygekasselaegernes Organisations Laboratorium, Copenhagen, for his help in referring patients, and to Dr J R. Rubini, V A. Hospital, Dallas, Texas, for performing the studies of BAIBA excretion.

This investigation was supported by PHS Research Grant CA 5492 HEM from the National Cancer Institute, U S. Public Health Service.

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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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Protein Pattern of Cerebrospinal Fluid During the Course of Acute Polyradiculoneuropathy[1]

By

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In 1859 Landry (14) reported 10 cases of acute flaccid paralysis which started distally in a limb and might develop into trunk, respiratory and pharyngeal paralysis. Paraesthesiae were also among the symptoms.

In 1916 Guillain *et al.* (10) described 2 cases with approximately the same symptoms. They also noticed that the total proteins in the cerebrospinal fluid (CSF) were increased without any accompanying increase of the cell content, so-called albumino-cytological dissociation.

In 1936 Guillain (11) reported a further 10 cases, but he did not mention in what stage of the disease the increase of proteins occurs. In 1938 van Bogaert *et al.* (3) noticed that the increase of proteins did not always occur in the first few days of the disease, but usually reached a peak after 25 to 45 days. According to Larsen and Fog (15) the increase of CSF proteins did not occur until the eighth day of the disease. Thus, in order to record the increased proteins it is not sufficient to make one single investigation of CSF, repeated puncture often being necessary. In one case described by Haymacker and Kernohan (12) the concentration of the total proteins on the second day of the disease was 20 mg/100 ml and on the eighth day 250 mg/100 ml. In another case the protein content fell from 114 mg/100 ml on the third day of the disease to 55 mg/100 ml on the sixth day. It is noteworthy that the concentration of total proteins is said to be normal or only slightly increased in the cisternal fluid, but considerably increased in the lumbar fluid. Aring (1) describes 3 cases. On comparison between lumbar fluid and cisternal fluid the findings were 330 and 14 mg/100 ml, 380 and 88 mg/100 ml and 1 400 and 82 mg/100 ml.

In the light of the reports referred to above the question arises whether polyradiculoneuropathy with and without increased proteins in the CSF represent two

Submitted for publication September 30 1963.

[1] Based on a lecture held before Svenska Läkaresällskapet on Dec. 1 1962.

different types of disease Haymacker and Kernohan (12) have given a detailed review of the literature. They have studied 50 fatal cases with pathological examination and made a total protein determination in 33 of these cases. Twelve cases had normal values. At autopsy they did not find the alterations of the nervous tissue in the group with elevated total protein values in the CSF to differ from those with normal values They concluded that there are not two separate groups of diseases and therefore recommended the term the Landry Guillain Barré syndrome.

Thus, determination of the total proteins of the CSF is not always sufficient to diagnose the disease. Moreover this method provides only limited possibilities of following the course of the disease. The purpose of the present work was to investigate whether immuno-electrophoresis of the proteins in CSF can be of diagnostic and prognostic value. An immuno-electrophoretic study of the changes in the pattern of the single fractions may offer greater possibilities of finding abnormalities, which are not reflected in the total protein value.

Methods

In this work we have only analysed CSF obtained by lumbar puncture. The total protein content was determined according to a modification of the method described by Waddell (22) Paper electrophoresis was performed by the method of Laurell *et al.* (16) The process of concentration of CSF and of micro-immuno-electrophoresis has been closely described in an earlier work (7) As immune serum we used a polyvalent antiserum which was obtained by immunizing rabbits with pooled sera from patients with marked serum abnormalities. The precipitation lines have been identified through the analyses of the mobility and the shape of the arcs, which are developed by the immune serum mentioned above, as well as with the aid of specific immune sera against α_2-macroglobulin and fibrinogen from Behringwerke.

As described earlier (7 21) in neurological diseases the CSF usually contains serum proteins not occurring in normal CSF These proteins are interesting since their presence in CSF must be considered an indication of abnormalities in the central nervous system. In this work the following fractions were studied particularly

α_2 macroglobulin. This protein, with a molecular weight of 850,000 is sometimes present even in normal CSF resulting in a weak precipitation arc. In CSF from cases with neurological diseases, however there is often a marked precipitate of the same appearance as that in serum and findings of this type are called positive.

β_1-lipoprotein. This protein with a molecular weight of 1,300 000 is never demonstrated in CSF samples from normal persons. It is best demonstrated with lipid staining (Sudan black) a technique described in an earlier work (7)

Transferrin of the type seen in serum. In immuno-electrophoresis of normal CSF two connected precipitation arcs appear while serum gives rise to only one arc. In CSF from some cases with neurological diseases transferrin of the type demonstrable in serum appears, that is a precipitation of only the anodic arc, called transferrin I Because of the characteristic shape of this precipitate there was no reason to use a specific immune serum.

β_2-macroglobulin. This is a large-molecular protein which has only been demonstrated in CSF from patients with a serious neurological disease. This precipitate has been demonstrated with an immune serum from a rabbit, which had developed antibodies against β_{2A} and β_2 macroglobulin but not against γ-globulin.

γ-globulin. In normal CSF the anodic part of the precipitation line of this protein is missing or occurs only in the form of a weak precipitate This part of th γ-globulin line was, however found to be marked in many patients with neurological diseases and is therefore recorded as a special pathological finding

Fibrinogen. This protein is never seen in normal CSF with the aid of the Ouchterlony or the immuno-electrophoretic methods.

Table I. The clinical condition, total proteins and immuno-electrophoretic findings of 12 patients with polyradiculoneuropathy

Sex and age	Day of disease	Condition	Cerebrospinal fluid						
			Total protein (mg/100 ml)	α_2 macro	β lipo	Transferrin I only	β macro	Marked anterior γ	Fibrinogen
H.P ♂ 34	9	Paresis of legs and N. facialis	464	+	+	+	+	+	+
R.P ♂ 19	6	Paresis of limbs, trunk, pharynx and N. facialis	354	+	+	+	+	+	+
T.P ♂ 63	4	Paresis of limbs and trunk	248	+	+			+	+
K.O ♂ 29	9	Paresis of limbs and trunk	234	+	+	+	+	+	+
J.A. ♂ 80	90	Paresis of limbs	180		+		+	+	+
R.H. ♂ 44	270	Paresis of limbs and trunk	132					+	+
G.H. ♀ 16	7	Paresis of limbs and trunk	129	+		+	+	+	
J.H. ♀ 46	14	Paresis of limbs and N. facialis	114	+	+		+	+	+
H.G.N. 18	10	Weak paresis of limbs and trunk	107		+			+	+
K.N ♂ 19	2	Weak paresis of legs	61		+			+	+
H.J. ♀ 55	7	Paresis of limbs and trunk	43		+			+	
N.P ♂ 32	3	Paresis of limbs and trunk	40		+			+	(+)

Material

During the last three years we have investigated and followed 12 patients with acute polyradiculoneuropathy, 10 of them from the Department of Infectious Diseases and 2 of them from the Department of Neurology, University Hospital, Lund. These patients had shown a typical clinical picture with generally extensive paralysis. Most of the patients were males (9 cases out of 12), a sex ratio agreeing with that found by Petlund (19). The patients' ages ranged from 16 to 80 years. Whenever possible CSF was obtained from these patients by lumbar puncture throughout the course of the disease, from many of them until recovery. Two patients died during the course of observation. One of them died from cardiac infarction, 80 years old, the other from gastric ulcer probably induced by steroid therapy.

Results

The 12 patients with polyradiculoneuropathy are summarized in table I. The first column gives sex and age, the next column gives day of the disease when the first lumbar puncture was performed, counted from the onset of the neurological symptoms. In two of the patients it was not possible for us to make investigations until after 90 days and 270 days. These two patients had still rather marked clinical symptoms with paralysis of the limbs and one of them had paralysis of the trunk. In the other 10 patients lumbar puncture was done within the first two weeks, 6 of them within the first week. The clinical state of the patients at the time of the first puncture is summarized with a note of the extent of the paralysis in the third column (table I). All patients had paralysis of the legs at the time of the first puncture and eight of them also had trunk paralysis. In three cases the facial nerve had been affected and one patient had pharyngeal paralysis as well.

Table II The clinical course total proteins and immuno-electrophoretic findings of a patient with acute polyradiculoneuropathy with late persistent symptoms

N P ♂ 32		Therapy	Cerebrospinal fluid						
Date	Condition		Total proteins (mg/100 ml)	α_2-macro	β lipo	Transferrin I only	β macro	Marked anterior γ	Fibrinogen
10/11—1961	Diarrhoea, fever								
14/11	Paresthesiae and weakness of limbs								
15/11	Admitted to the hospital		40		+			+	(+)
	Paresis of limbs and trunk								
17/11		Steroids							
18/11	Total paralysis	Tracheotomy Artif. resp.							
22/11	Beginning regression of paralysis		155	+	+	+	(+)	+	(+)
20/12	No pharyngeal paresis		130		+	+			+
9/1 —1962	Out of respirator		125	+	+	+			+
7/2	Slight improvement		84		+	+		(+)	+
	Sitting 2 to 3 hours/day								
8/3	Sitting 4 to 5 hours/day		72		(+)	+		+	+
5/4	Beginning regression of paralysis of arms		68					(+)	
4/5	Beginning regression of paralysis of legs		62						
26/5	Very slow regression but	No	55						
23/7	not completely recovered	steroids	43						
30/11	at time of last puncture		47						

Cerebrospinal fluid

The total protein values in the CSF were usually increased at the time of the first lumbar puncture. Only three patients, who were punctured very early have shown normal or relatively normal total protein values. When re punctured only a few days later two of them showed increasing values, 40 to 170 mg/100 ml and 61 to 167 mg/100 ml Unfortunately it was not possible for us to re puncture the third patient with initially normal total proteins in the CSF on the seventh day of the disease.

Thus with the exception of this last mentioned case, all showed increased total protein in the CSF sooner or later during the course of the disease On frequent re-punctures during the acute stage of the disease the maximum value occurred during the first three weeks (table III)

Analysis with paper electrophoresis of CSF showed only slight variations of the protein fractions, generally within the normal limits.

The last six columns of table I give a survey of the immuno-electrophoretic findings from the first puncture of the pro-

Table III The clinical course, total proteins and immuno-electrophoretic findings of patient with benign polyradiculoneuropathy (see fig. 1)

G. H. ♀ 16		Therapy	Cerebrospinal fluid						
Date	Condition		Total protein (mg/100 ml)	α_2-macro	β_1 lipo	Transferrin 1 only	β_1 macro	Marked anterior γ	Fibrinogen
8 11–1962	Myalgia								
10 11	Tired; stayed in bed								
12 11	Paresis of legs								
13 11	Admitted to the hospital Paresis of limbs and trunk		129	+		+	+	+	
16 11	Paresis of pharynx	Steroids Tracheotomy Artif. resp							
17 11	Out of respirator								
20 11	Beginning regression of paralysis No pharyngeal paresis		160	+	+	+	+	+	+
30 11	Returning function of arms		210	+	+	+	(+)	+	−
21 12	No paresis tired		42					+	
18 1 –1963	No symptoms and signs of the disease	No steroids	44					+	

terns discussed above. As shown in this table, the β_1-lipoprotein, fibrinogen and an increased amount of anodic γ-globulin are the most common pathological findings. These findings in the CSF correspond well t observations in other neurological diseases (6, 7 21) in which the three fractions mentioned occur in obviously mild disorders of the blood/CSF barrier. β_1-macroglobulin and transferrin of the type seen in serum are less common findings in CSF as well as a marked precipitate of α_2-macroglobulin. It must be observed that the three of our patients with total protein values between 40 and 61 mg/100 ml in the acute stage showed boormal immuno-electrophoretic findings.

In tables II and III two separate cases are summarized with short notes of the clinical progress and therapy. The tables also include determinations of the total proteins and the immuno-electrophoretic findings. The course of these two cases can be considered representative of the material as a whole. As the patient recovers, the findings in the CSF become normal. In three of the cases, however a marked anodic γ-globulin fraction persisted even when the patient had no clinical symptoms. In two of the twelve cases, on the other hand, the findings in the CSF became normal in every respect even in the absence of clinical recovery. In these cases the last investigation was performed more than six months after

Table II. The clinical course, total proteins and immuno-electrophoretic findings of a patient with acute polyradiculoneuropathy with late persistent symptoms

N. P. ♂ 32		Therapy	Cerebrospinal fluid						
Date	Condition		Total proteins (mg/100 ml)	α_2 macro	β lipo	Transferrin I only	β_1 macro	Marked anterior ?	Fibrinogen
10/11—1961	Diarrhoea, fever								
14/11	Paresthesiae and weakness of limbs								
15/11	Admitted to the hospital		40		+			+	(+)
	Paresis of limbs and trunk								
17/11		Steroids							
18/11	Total paralysis	Tracheotomy Artif. resp.							
22/11	Beginning regression of paralysis		155	+	+	+	(+)	+	(+)
20/12	No pharyngeal paresis		130		+	+			+
9/1 —1962	Out of respirator		125	+	+	+			+
7/2	Slight improvement		84		+	+		(+)	+
	Sitting 2 to 3 hours/day								
8/3	Sitting 4 to 5 hours/day		72		(+)	+		+	+
5/4	Beginning regression of paralysis of arms		68					(+)	
4/5	Beginning regression of paralysis of legs		62						
26/5	Very slow regression but	No steroids	55						
23/7	not completely recovered		43						
30/11	at time of last puncture		47						

Cerebrospinal fluid

The total protein values in the CSF were usually increased at the time of the first lumbar puncture. Only three patients who were punctured very early have shown normal or relatively normal total protein values. When re-punctured only a few days later two of them showed increasing values, 40 to 170 mg/100 ml and 61 to 167 mg/100 ml. Unfortunately it was not possible for us to re-puncture the third patient with initially normal total proteins in the CSF on the seventh day of the disease.

Thus, with the exception of this last mentioned case all showed increased total protein in the CSF sooner or later during the course of the disease. On frequent re-punctures during the acute stage of the disease the maximum value occurred during the first three weeks (table III).

Analysis with paper electrophoresis of CSF showed only slight variations of the protein fractions, generally within the normal limits.

The last six columns of table I give a survey of the immuno-electrophoretic findings from the first puncture of the pro-

Discussion

The total CSF protein value varies during the course of acute polyradiculoneuropathy which necessitates frequent punctures, especially during the initial phase of the disease, if pathological values are to be demonstrable. The results obtained in this study indicate that the increase of total CSF proteins reaches its height within the first three weeks of the disease. The increase of the total proteins at this stage of the disease are apparently caused by an increased passage of plasma proteins through the blood/CSF barrier

In the three cases, which had normal or relatively normal CSF protein values at the time of the first puncture, immunoelectrophoresis showed two or more protein fractions not normally occurring in CSF

In spite of its name, acute polyradiculoneuropathy often lasts a long time. The frequent lumbar punctures performed in this study made it possible to follow up the changes of the CSF and to compare them with the clinical observations. Several patients were followed until they had definitely recovered and returned to work. As the patients recovered the separate protein findings in the CSF usually became normal. The lack of agreement between the clinical picture and the CSF findings in the two cases with persisting paralysis may be explained by a compression damage of the spinal roots and thus not by persisting activity of the disease

In three of the twelve cases, however marked anodic γ-globulin fraction persisted even when the patients had made a clinical recovery The question arises why this abnormal CSF protein finding is the last one to disappear during the course of otherwise uncomplicated acute polyradiculoneuropathy Together with certain β_{2}-globulins — β_{A} and β-macroglobulin — γ-globulin is involved in the immune defence and these proteins have therefore been termed immune globulins. Apparently the γ-globulin can be produced locally within the central nervous system, an opinion shared by several authors (2 5 8, 9 13, 17 18)

According to an alternative opinion all the γ-globulin in the CSF originates from the serum. The appearance of an increased anodic γ-globulin fraction in the CSF may then be a result of an increased serum concentration of this protein. This opinion is supported by the findings in patients with myeloma and cirrhosis, who show a similar protein proportion of abnormal γ-globulins in CSF and serum in cases without any signs of a disturbed blood/CSF barrier (20) Using the dilution technique discussed earlier we were not able to demonstrate any increase of the anodic γ-globulin portion in the serum from two of the three above-mentioned cases with increased amounts in the CSF Thus, the increased amounts in the CSF may be the result of a local overproduction of immune globulins in the central nervous system. Such a production of γ-globulin within the central nervous system apparently also occurs often in, for example, multiple sclerosis.

Immuno-electrophoretic identification of separate proteins in the CSF as postulated in the beginning has provided better possibilities of studying the changes during the course of the disease than the determination of total CSF proteins and paper electrophoresis only The often irregular course of acute polyradiculoneuropathy and the disproportion between total CSF proteins and clinical symptoms, discussed by many authors,

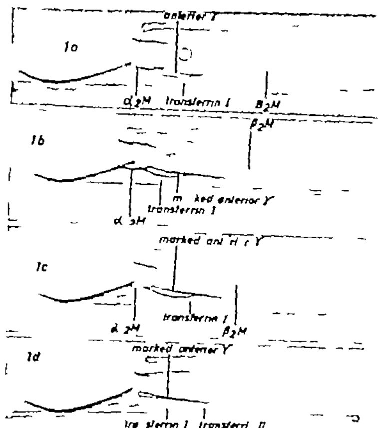


Fig 1 Immuno-electrophoresis of CSF of the patient referred to in table III

a) 15 11 62 Observe the presence of β_2-macroglobulin, a very marked precipitate, of α_2-macroglobulin, transferrin of serum type (thus absence of the CSF specific second transferrin arc) and a marked *precipitate of the anodic part of the* γ-globulin. On this and subsequent occasions the lower basin contains a polyvalent immune serum against serum and the upper basin contains an immune serum from a rabbit that did not develop antibodies against γ-globulin. The preparation was stained with amido black *10 B*.

b) 20 11 62. The CSF now shows all 6 pathological findings. The fibrinogen was studied separately with anti-fibrinogen serum. The β_1-lipoprotein does not appear on amido black staining but it does show up on lipid staining

c) 30 11 62 The β_2-macroglobulin occur only as a weak precipitate.

d) 21 12 62. All pathological findings except a marked anodic part of the γ-globulins have now disappeared.

the onset of the disease. One of the two patients had still extensive paralysis, the other still had weakness of the arms.

Serum

Determination of the amount of total proteins and analysis with paper electrophoresis have been performed parallel with the investigations of the CSF and they have only shown insignificant variations In some cases an increase of the α globulin fraction and a moderate reduction of albumin were noted i e common findings in acute processes.

Serum was also analysed by the immuno-electrophoretic technique. By the dilution method we have tried to estimate the amount of γ-globulin in serum collected on different occasions in the course of the disease. By this method Clausen (4) found a parallel increase of immune globulins in CSF and serum in multiple sclerosis in acute but not in stationary cases and a suspected correlation in infectious diseases in the central nervous system. With the dilution method we were only able to demonstrate an increased amount of the anodic part of the γ-globulin in serum in three cases. Such a finding however was not made in *two of the three patients with an increased* amount of the anodic part of the γ-globulin in CSF after clinical recovery

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

From the Medical Clinic B (Renal Clinic) University of Lund, Sweden

Treatment of Exogenous Poisoning with Special Regard to the Need for Artificial Kidney in Severe Complicated Cases

By

KARL ERIK HAGSTAM and TORE LINDHOLM

Over the 5-year period 1958—1962, 150 patients were on 161 occasions treated at this clinic for exogenous poisoning. In this presentation the series is considered to consist of 161 "cases". The series includes 50 selected cases of severe intoxication. 26 (16 %) were referred for special care from hospitals within other regions and 24 (15 %) from hospitals within our own region. The remaining 111 cases were admitted direct to this clinic.

Incidence, age and sex

It will be seen from table I that the annual number of cases rose gradually from 15 in 1958 to 53 in 1962. The length of hospital care for these cases increased correspondingly. The average number of bed-days remained constant, being around 8.

Fig. 1 shows the series divided into age groups (10—19 years, and so on, up to 70—79 years) and the distribution by sex within the groups. The age-group 20—29 years is the biggest, comprising one-third of the series. 85 cases (52) were females.

Aetiology

In the majority of cases the poisonings were suicide attempts. Demonstration or "mistake" could have been present in 57 cases (35 %) and serious intention in 73 (45 %). In 31 cases (19 %) the intention could not be established. 39 cases (24) had earlier been treated at this or some other clinic for intentional poisoning.

Table II shows the distribution of the cases according to the substance (drug etc.) used to produce the poisoning. Some cases of mixed poisoning are included in more than one group. Barbiturates had been used in 48 and meprobamate in 17 % of the cases.

Table III shows the duration of "unconsciousness" in cases in which mental function was depressed to such a degree that the patient did not respond when spoken to and could not take any fluid. In some cases, for instance in digitalis and mercury intoxications, such depression did not occur at all. The table also shows the distribution of respiratory and dialysis treatments, both of which were given in 21 cases. In the evaluation of this table, the fact that dialysis treatment often shortened the period of unconsciousness must be taken into account.

Treatment other than dialysis

) Gastric lavage was done in 13 cases (8 %) of poisoning with tablets, while the patient was still awake. In 2 of these cases, however, severe intoxication (unconsciousness for 6 days) could not be prevented. Gastric lavage gives a poor

Submitted for publication September 30, 1963

emphasizes the requirements of new methods for analysis of the CSF

Summary

Acute polyradiculoneuropathy or the Landry-Guillain Barré syndrome is a disease with suddenly occurring motor and sensory symptoms and it has been shown that it is generally combined with an increase of the total CSF proteins. A material of 12 patients has been reported and the clinical courses of the disease and the changes of the CSF have been followed up over a long period. The CSF was studied by conventional methods as well as by micro-immuno-electrophoresis with the aid of different immune sera and staining methods. Interest has been concentrated mainly on certain protein findings normally not occurring in CSF. The following principal observations are made

1 During the acute stage of the disease there is almost always an increase of the total proteins in CSF but not always in the first few days. The increase is however always greatest within the first three weeks.

2 Even if the concentration of the total proteins is not increased initially proteins usually not occurring in CSF appear in this stage most likely as a sign of a disorder of the blood/CSF barrier

3 Repeated lumbar punctures show that the immuno-electrophoretic findings in CSF usually become normal almost linearly with the clinical picture.

Acknowledgements

This investigation was supported by grant from Alfred Österlund' Foundation and Riksföreningen mot polio.

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of bronchopneumonic type and varying extension.

d) Respirator treatment was necessary in 45 cases (28 %) in 15 because of respiratory standstill.

e) In all the cases with normal renal function large amounts of fluid were given, with frequent checkings of the electrolyte-fluid balance (urinary output, serum-electrolytes, body-weight, and chest X-ray). A treatment adopted in the last 3—4 years to force diuresis was intravenous administration of urea solution (9a). This procedure was preferentially used in barbiturate intoxications, but was also applied in cases in which the poisoning was caused by for instance, meprobamate and other substances that are easily eliminated via the kidneys.

f) A tendency to shock was treated by administration of nor-epinephrine and/or metaraminol in 54 cases (34 %).

Table II

Type of poisoning	No. of cases
Pentymal	19
Allypropymal	2
Phenobarbital	7
Dexmal	2
Barbiturates other than above (total 77)	47
Meprobamate	27
Phenothiazine derivatives	7
Piperidinedione derivatives	3
Bromides	2
Morphine	1
Antihistamines	1
Acetylsalicylic acids	9
Methyl alcohol	6
Ethyl alcohol	11
Ethylene glycol	1
Chlorinated hydrocarbons	2
Mercury compounds	4
Digitalis	2
Isoniazid	1
Carbon monoxide	6
Others	16

Dialysis treatment

Seventy-three haemodialyses were performed in 30 cases with the artificial kidney of Alwall type. Seven patients were dialysed because of uraemia (acute renal failure as a complication of the intoxication). In the remaining 23 cases the artificial kidney was used for the purpose of removing the toxic substance. The distribution of these 23 cases according to the substance that produced the intoxication is shown in table V.

The barbiturate cases predominate, amounting to 13. Earlier (1952—1957) a further 7 patients were treated with the artificial kidney because of barbiturate poisoning. This brings the clinic's total number of such cases to 20 and the total number of dialyses to 27.

Some case histories will illustrate the effect of dialysis.

A 71-year-old woman (fig. 2) was admitted unconscious to another hospital after having taken an unknown amount of barbiturates. Because of deterioration of general condition she was transferred to this clinic on the third day. On admission she had severe hypoxia and carbon-dioxide retention as a result of stagnation of secretion and bilateral pneumonia. After intubation and aspiration to keep the air passages free, respirator treatment was started; later on the same day tracheotomy was performed. The barbiturate concentration in the serum was 30 mg/100 ml. Administration of large amounts of fluid did not increase the urinary output, possibly because of temporarily impaired renal function. On day 4 she was dialysed for 8 hours. During the dialysis the barbiturate content in the serum fell from 35 mg to 12 mg/100 ml (by 66 %). As the patient was still unconscious on day 6, a second dialysis treatment was given, and the barbiturate level fell from 15 mg to 7 mg/100 ml (by 53 %). In

Table I

Year	No. of cases	No. of bed-days	Mean length of hospital care (days)	Total no. of bed-days at the clinic	Percentage of cases of poisoning
1958	15	125	8.3	12,485	1.00
1959	17	135	7.9	12,958	1.04
1960	24	182	7.6	13,661	1.33
1961	50	412	8.2	15,219	2.71
1962	55	395	7.2	14,514	2.72

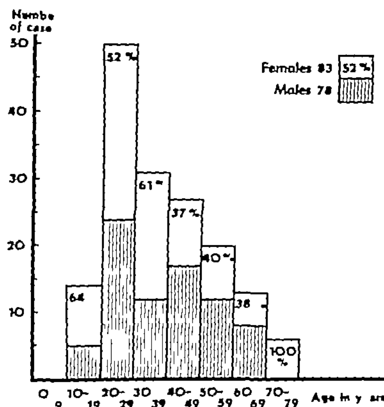


Fig 1

result and should be replaced by aspiration and/or emetics (5)

b) Antidotes were given in 6 cases (4 %) Nalorphin in morphine poisoning and BAL in cases of mercury poisoning

c) Intubation and/or tracheotomy were carried out in 75 cases (47 %) (table IV) This relatively high figure is explained by the selection of severe cases which could not be treated in other hospitals The intubation was followed by tracheotomy after 24 hours if the patient did not wake up The tracheotomy tube could be removed within 6 days in 22 cases and was needed for more than 6 days in 21 cases.

Chest radiographs were taken in all cases in which the patient was unconscious for 24 hours or more. In 40 cases (25 %) there were pulmonary changes

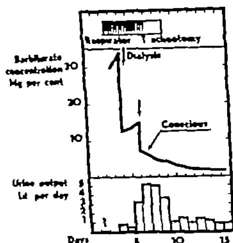


Fig. 2.

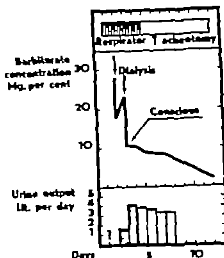


Fig. 3.

a long-acting barbiturate compound and/or acute renal failure, and in patients with complications such as pneumonia or respiratory standstill. The dialysis treatment helped to shorten the period of unconsciousness. Similar observations have been made by Alwall et al. (2) Berman et al (3) Honey and J ckson (6) Nakamoto and Kolff (10) Jorgensen and Wieth (7) and others. The concentration of barbiturate n serum t the beginning of dialysis averaged 15 7 mg/100 ml (range 1 6—63 mg) and could be lowered by an a erage of 41.5 (range 11.5—88) during 6 to 8 hours of dialysis

Fou cases of methyl-alcohol poisoning were treated by dialysis. These cases will be published by Erlanson et al. in Acta med scand.

Poisoning with ethylene glycol oc curred in 1 case which will be described by Hagstam et al. in Acta med. scand.

Our series includes 1 case of isoniazid poisoning. The course was that charac teristic of such poisoning (9)

A 33-year-old woman with pulmonary tuberculosis, which was treated with isoniazid, took bout 40 g of isoniazid for suicidal purposes. She was admitted to hospital after 1 hour and was then able to answer when spoken to but became quickly unconscious, with generalized convulsions which were brought under control with Isomyl-natrium intravenously She had profuse salivation and, at first, deep respiration. After another hour respiratory standstill occurred. She was tracheotomized and treated in respirator Checking of electrolytes showed a marked metabolic acidosis with corrected bicarbonat of 8.0 mMol/l and pH 6.87 Repeated generalized convulsions were controlled by curarisation. Her condition remained unchanged until day 3 After exchange transfusion spontaneous breathing returned. During transportation to this clinic respira tory standstill recurred and necessitated artificial respiration. By dialysis treatment the isoniazid concentration in the serum was re duced from 6.8 mg to 3.0 mg/100 ml. Her condition did not improve, however she died on the following day

40 g of isoniazid exceeds the dose stated to be lethal (9) One dialysis treat ment reduced the concentration in the serum by 56 in our case Katz and Car

Table III

Unconsciousness (days)	No. of cases		Respirator treatment		Dialysis treatment	
	Survivals	*Deaths*	*Survivals*	*Deaths*	*Survivals*	*Deaths*
<1	60	0	0	0	4	0
1	46	1	5	1	3	0
2	16	2	8	1	2	2
3	10	2	8	2	3	2
4	10	1	7	1	4	1
5–9	8	5	7	5	5	4
Total	150	11	35	10	21	9

Table IV

	Male	Female	Total	
Intubation alone	12	20	32	Intubations total 62
Intubation and tracheotomy	12	18	30	
Tracheotomy alone	9	4	13	Tracheotomies total 43
Total	33	42	75	

Table V. Dialysed cases. Indication: poisoning

Toxic substance	No. of cases
Barbiturate	15
Methyl alcohol	4
Ethylene glycol	1
Isoniazid	1
Mixed poisoning	4
Total	25

response to intravenous administration of urea and fluid, polyuria started on day 3. During the second dialysis urea was also added to the dialysing fluid. On day 7 the patient woke up. After another two days the tracheal tube was removed. The patient was then transferred to a psychiatric department.

A 51 year-old man (fig. 3) was admitted to this clinic deeply unconscious, without pupillary and tendon reflexes, about 12 hours after having taken at least 9 g of phenobarbital. His blood-pressure was 70/50 mm Hg, and nor-epinephrine was added to the fluid administered intravenously. Respiratory failure necessitated tracheotomy and respirator treatment. The concentration of phenobarbital in the serum was 28 mg/100 ml. A 10-hour dialysis and forced polyuria reduced the level to 18 mg (by 37). The sleep became more superficial and the tendon reflexes returned. On the next day the phenobarbital concentration was 23 mg/100 ml; a second dialysis reduced it to 10 mg (by 57). Towards the end of the dialysis the patient began to breathe and made spontaneous movements. On the following day he woke up, and the respirator was no longer necessary. Treatment by forced diuresis was continued for a few days more. Because of purulent tracheobronchitis the tracheal tube was not removed until one week after return of consciousness. On the same day the patient was transferred to a psychiatric department for further care.

The usual treatment of barbiturate poisoning was supplemented by dialysis in patients who had a high serum level of

A 25-year-old man (fig. 4) who had taken mercuric chloride for suicidal purposes, was admitted to hospital after about 9 hours and was at once given dimercaprol (BAL). Immediately after his arrival at the hospital the patient vomited bloody matter twice and had blood-pressure fall which was quickly offset by administration of fluid. In the first 24 hours the urinary output was satisfactory (1 400 ml), but thereafter anuria developed and he was therefore transferred to this clinic. His general condition was good throughout, and no further symptoms referable to the gastrointestinal tract appeared. By the ninth day N.P.N. had risen to 227 mg/100 ml; one treatment with the artificial kidney was carried out without complications and N.P.N. was reduced to 95 mg/100 ml. On days 14 and 15 N.P.N. was again more than 200 mg/100 ml, but as diuresis started it fell and became normal again on day 22. In connection with the start of urine production the patient was given more dimercaprol (BAL).

The renal damage that occurs in poisoning with mercury compounds is reversible. Patients with such poisoning who show signs of impending uraemia should be treated with the artificial kidney. Successfully treated cases of this kind have been reported by, for instance, Alwall (1), Noelle (11) and Válek et al. (12).

In the remaining 5 patients who were treated with dialysis, acute renal failure occurred after shock. The substances taken were barbiturates in 2 cases, chloralose in 1, carbon monoxide in 1 and meprobamate plus carbon monoxide in 1 case. Two of these patients (nos. 5 and 6, table VI) died and 3 survived.

Outcome

Of the 161 cases in the series, 11 (7 %) were fatal. The substances taken and the causes of death are summarized in table VI.

Table VI

Deaths	Toxic substance	Cause of death
1	Methyl alcohol	Cerebral damage
2	Methyl alcohol	Cerebral damage
3	Methyl alcohol	Cerebral damage
4	Isoniazid	Cerebral damage
5	Chloralose	Cerebral anoxia, haemorrhagic diathesis
6	Barbiturate	Infected frost-bites with septicaemia after recovery from poisoning
7	Dinusal	Cerebral anoxia before admission. Recovery from poisoning
8	Several poisons including meprobamate	Acute heart-failure 40 min. after admission
9	Several poisons including meprobamate	Acute heart-failure 90 min. after admission. Cardiac massage after thoracotomy. Death 21 days later from cerebral damage
10	Several poisons including phenothiazine derivatives	Septicaemia (Ps. pyocyanea)
11	Unknown	Generalized toxic organic damage

Among the 150 survivals, 65 were transferred to psychiatric clinics and 25 to other clinics. Sixty cases could be discharged to their homes.

Summary

Over the 5-year period 1958—1962 150 patients were on 161 occasions ("cases") treated for exogenous poisoning at this clinic. Eleven cases (7 %) died

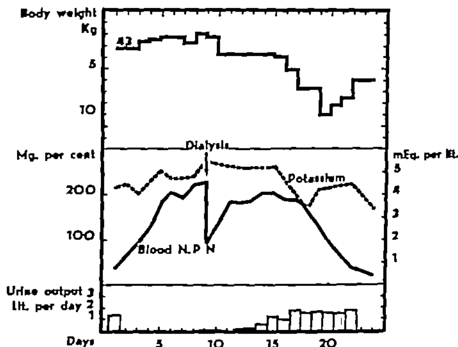


Fig 4

ver (8) in a child with isoniazid poisoning carried out exchange transfusion with a good effect. Jorgensen and Wieth (7) reported a dialysis-treated case of isoniazid poisoning. As no specific antidote exists, patients with severe isoniazid poisoning should be treated with the artificial kidney as promptly as possible.

The 4 patients in the group of mixed poisoning were all under treatment for mental depression and had access to several psychotropic drugs (meprobamate, phenothiazine derivatives, Librium® etc.) These patients were severely intoxicated and the artifical kidney was used in an attempt to control the poisoning. Two patients survived and two died (nos. 9 and 10, table VI). In one case determination of the meprobamate concentration in serum was made. By one dialysis it was reduced from 17 mg to 10 mg/100 ml (by 41 %). In a few cases of meprobamate poisoning dialysis treatment has been considered (4), but we have not been able to find any published case in which such treatment was carried out. Meprobamate is dialysable, and treatment with the artifical kidney can therefore be indicated in cases of severe poisoning with this substance.

Among the 23 patients dialysed on the indication of poisoning 5 had severe acute renal failure which necessitated further dialysis because of uraemia. Of these 5 patients 1 had barbiturate poisoning, 1 ethylene-glycol poisoning, and 3 mixed poisoning. In 4 of them renal function returned. 1 patient died anuric (no. 10, table VI).

Besides these 5 patients, 7 were treated with the artifical kidney on the indication of uraemia.

The series includes 4 cases of mercury poisoning (table II). 2 of them acquired renal damage with long-continued uraemia which necessitated dialysis treatment. In both these cases the course was similar, one will be described here.

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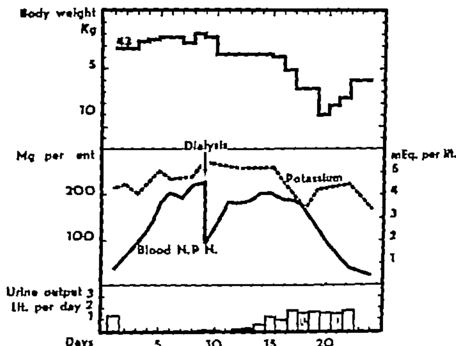


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Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

From the Central Laboratory Kommunehospitalet, Århus, Denmark

Studies on the Osmotic Fragility of Normal Human Erythrocytes

V The Concentration of Glutathione During Incubation and its Relation to Changes of the Osmotic Fragility

By

Esper Mortensen

A close correlation has been described between the concentration of reduced glutathione (GSH) and the life span of erythrocytes from patients suffering from glucose-6-phosphate dehydrogenase deficiency (3) As shown by Lohr et al. (10) the activity of glucose-6-phosphate-dehydrogenase decreases with the in vivo and in vitro age of normal human erythrocytes. The osmotic fragility of erythrocytes is known to increase with the in vivo as well as the in vitro age of the cell (8, 11 12,17) The glutathione stability test is generally accepted to reflect the glucose-6-phosphate-dehydrogenase activity (4) In previous paper the changes of osmotic fragility during various conditions of incubation were described (12) The present experiments were designed to investigate the concentration and stability of glutathione of normal human erythrocytes during incubation with variation of the experimental conditions, as in the investigations of the osmotic fragility in order to describe their interrelationship and correlate the changes found to the consumption of glucose.

Submitted for publication September 30, 1963.

Material and methods

Blood from healthy voluntary blood donors was used throughout the investigations. Determinations of the concentration of hemoglobin, the erythrocyte count, the leucocyte count, the reticulocyte count, the differential leucocyte count and hematocrit were made on all samples by conventional methods.

The concentration of reduced glutathione was determined according t the method previously described (13)

The stability test was performed with menadione. In order to obtain distinct expression for the possible variations of the stability of GSH of the erythrocytes during incubation, the influence of the menadione concentration, the temperature during the incubation with menadione, and the time of incubation with menadione were investigated. Fig. 1 shows the elation between the concentration of menadione and the time of incubation — and the decrease of the concentration of reduced glutathione. The temperature during incubation with menadione for 2 hours did not influence the decrease of the concentration of GSH.

The solutions of menadione are stable for t least one week when stored in the refrigerator

I contrast to the stability test with acetylphenylhydrazine (3) no effect of oxygenation

The series includes 50 selected cases of severe poisoning referred from other hospitals for special care.

The poisons used were barbiturates in 48 %, meprobamate in 17 %, other psychotropic drugs in 9 %, acetylsalicylic acid in 6 %, methyl alcohol in 4 %, ethyl alcohol in 7 %, mercury compounds in 2 %, carbon monoxide in 4 % and others in 15 %.

Intubation and/or tracheotomy was indicated in 75 cases (47 %) and respirator treatment in 45 (28 %). Patients poisoned with substances that are eliminated by excretion in the urine were if possible treated by forced diuresis.

Thirty cases were treated with the artificial kidney altogether 73 times. In 23 of these cases haemodialysis was performed in order to remove the toxic substances: 13 of them had barbiturate poisoning, the others had poisoning with methyl alcohol, ethylene glycol, isoniazid, meprobamate and/or other drugs. Some case histories are presented to illustrate the effect of dialysis. With our technique (dialyser of Alwall type) a dialysis treatment reduced the barbiturate content of serum on an average by 41.5 %.

In 12 cases the poisoning was complicated by severe acute renal failure which necessitated haemodialysis on the indication of uraemia. Two of these cases had mercuric chloride poisoning.

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Table II. The concentration of reduced glutathione (GSH) after incubation for 0 6 12 and 24 hours. Blood samples from 6 healthy donors were subjected to incubation at both temperature levels Values for GSH are mg/100 ml erythrocytes

No.	0 hr	6 hrs	12 hrs	24 hrs	No.	0 hr	6 hrs	12 hrs	24 hrs
A 5° C					*B* 25° C				
1 a	81	86	82	79	1	81	82	82	67
b	72	71	71	61	b	72	73	35	20
	87	76	66	47		87	53	30	13
2	78	80	78	74	2	78	78	75	67
b	71	68	67	32	b	71	65	17	13
	72	66	55	41		72	49	22	11
3	87	87	87	83	3	87	89	83	75
b	76	72	72	70	b	76	70	43	17
	83	78	62	53		83	53	30	10
4	83	81	81	77	4	83	83	82	67
b	70	72	64	43	b	70	69	13	13
	62	54	41	34		62	38	23	20
5	63	69	70	64	5	63	69	70	67
b	57	60	60	60	b	57	60	30	16
	83	74	68	53		93	63	38	10
6	73	75	73	73	6	73	73	77	67
b	63	67	63	57	b	63	65	45	17
	93	86	74	55		83	63	30	11
Mean	78	80	78	75	Mean	78	80	78	68
b	68	67	66	57	b	68	67	31	16
	80	72	61	48		80	33	29	12

= before stability test b = after stability test = blood sugar in mg %.

after incubation at 37° C for 0—6—12 and 24 hours A decrease of the con centration of GSH before the incubation with menadione to bout 50 % of the initial value gradually occurred during the period of incubation. Following the incubation with menadione the con centration of GSH rapidly fell eaching an lmost constant, low value after 12 hours. The concentration of glucose in the incubated blood reached the level for non-glucose reducing capacity at about the same time (table I)

b) *The temperature during incubation* The influence of varying the temperature was investigated by incubating samples of blood from the same donor at 5—25 and 37° C for 0—6—12 and 24 hours and determining the concentration and stabil ity of GSH of the erythrocytes t the times mentioned. The decrease of the concentration of GSH was obviously lessened by a lowering of the temperature. The stability of GSH was preserved far better during incubation at lower temperatures than at higher ones (table II)

c) *The concentration f glucose during the incubation.* The concentration of glucose in incubated blood seemed to be closely correlated to the concentration of GSH after the stability-test. In order to evaluate this further samples of blood from six

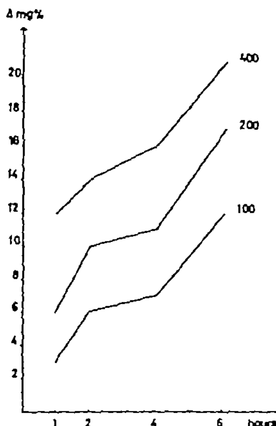


Fig 1 The relation between the decrease of the concentration of GSH of a blood sample (ordinate) and the time of incubation with menadione (abscissa) The figures at the right ends of the curves indicate the amount of menadione (menadione sodium bisulfit) added in μg/ml heparinised blood.

could be demonstrated with the menadione test.

According to the results obtained the final stability test was performed with 100 μg menadione/ml of heparinised blood incubated for 9 hours at 37° C. The concentration of glutathione was determined before and after the incubation.

The effects of the following variables during incubation of erythrocytes were investigated in relation to the concentration and stability of the GSH a) the period of incubation before the stability test, b) the temperature during this incubation, c) the concentration of glucose at the beginning of the incubation, d) the pH during the incubation.

In the experiments great care was taken only to use well mixed samples of blood Whenever the composition of a blood sample

Table I The concentration of reduced glutathione (GSH) after incubation for 0 6 12, and 24 hours at 37°C. Experiments were performed on blood from 6 healthy donors Values for GSH are mg/100 ml erythrocytes

No.	0 hr	6 hrs	12 hrs	24 hrs
1 a	91	83	79	52
b	74	22	17	15
c	107	40	21	18
2 a	90	90	73	44
b	73	21	14	15
c	83	27	15	15
3 a	83	83	71	41
b	68	21	14	15
c	80	23	17	15
4 a	73	74	57	30
b	62	12	11	13
c	78	26	19	15
5 a	85	85	75	43
b	74	23	19	14
c	82	34	14	14
6 a	58	59	48	30
b	48	12	14	13
c	71	24	19	14
Mean	80	79	67	40
b	66	19	15	15
c	84	29	19	15

— before stability test

b = after stability test.

c = blood sugar in mg%.

was varied untreated portions of the same blood acted as control. Details of the experimental technique can be obtained from the tables or the legends to the figures.

The concentration of glucose was determined by the method of Hagedorn et al. (7)

The pH and standard bicarbonate were measured according to the method of Andersen et al. (1)

Results

a) *The period of incubation.* The stability test was performed on parts of the same well mixed heparinised blood sample

Table IV. The concentration of glutathione (GSH) incubation for 0, 6, 12 and 24 hours at 37° C. Blood samples from 6 different healthy blood donors were used throughout the experiments. In A the samples were incubated after the addition of glucose and lactic acid, in B the samples were incubated after the addition of glucose and Na_2CO_3. Values for GSH are mg/100 ml erythrocytes

No.	0 hr	6 hrs	12 hrs	24 hrs	No.	0 hr	6 hrs	12 hrs	24 hrs
A					*B*				
1	83	83	83	81	1 a	83	83	80	74
b	69	69	68	69	b	64	64	24	14
c	236	211	165	74		216	170	115	21
d	16	12	9	6	d	32	23	19	14
2	82	78	78	67	2	82	78	80	49
b	64	64	64	20	b	60	60	13	13
	156	127	74	21		132	90	45	21
d	15	12	10	7	d	30	23	19	17
3	97	93	93	88	3	97	97	97	82
b	79	79	79	19	b	78	81	14	14
	183	160	105	24		137	114	41	19
d	15	12	9	6	d	27	21	17	15
4	82	84	80	62	4	82	84	82	56
b	67	68	67	16	b	64	84	24	16
	212	165	103	27		220	121	43	12
d	18	12	9	7	d	36	24	20	14
5	83	85	83	65	5 a	83	83	83	57
b	70	70	67	17	b	67	67	13	15
	197	130	74	15	c	201	98	34	12
d	17	12	9	7	d	37	23	19	17
6	81	79	79	65	6	81	81	84	42
b	67	70	65	16	b	65	65	16	16
	204	134	78	12		201	95	31	11
d	18	13	10	7	d	37	25	21	17
Mean	85	84	83	71	Mean	85	84	84	57
b	69	70	69	26	b	66	66	18	15
	198	154	99	28		188	115	52	16
d	16	12	9	7	d	37	23	19	16

– before stability test   b = after stability test   c = blood sugar in mg %   d = standard HCO_3

) Normal values. On the basis of the determinations of the concentration and stability of GSH of unincubated blood from 24 healthy donors, the following normal values were calculated: concentration of GSH = 84 mg/100 ml erythrocytes with a standard deviation of 8.5. The concentration of GSH after the incubation with menadione, i. e. after the stability test = 72 mg/100 ml erythrocytes with a standard deviation of 9.0.

On the basis of the determinations of the concentration and stability of GSH after incubation for 24 hours at 37° C of blood from 12 adult healthy donors, the following normal values were calculated: concentration of GSH = 44 mg/100 ml erythrocytes with a standard deviation of

Table III The concentration of reduced glutathione (GSH) after incubation for 0 6 12 and 24 hours at 37 C In A the glucose content of the blood was within normal limits. In B glucose was added to a concentration of 275—300 mg%. Blood samples from 6 healthy blood donors were used in the experiments Values for GSH are mg/100 ml erythrocytes

No.	0 hr	6 hrs	12 hrs	24 hrs	No.	0 hr	6 hrs	12 hrs	24 hrs
A					*B*				
1 a	53	49	46	30	1 a	53	53	48	46
b	45	15	11	10	b	45	45	4.	15
c	87	30	19	13	c	298	234	140	22
2 a	100	90	81	60	2 a	100	100	89	84
b	91	20	11	11	b	91	86	78	64
c	87	26	18	18	c	300	224	159	62
3 a	91	85	75	50	3 a	91	83	81	81
b	81	24	13	13	b	81	75	70	34
c	108	28	18	22	c	294	228	181	62
4	78	78	65	43	4 a	78	6	74	68
b	70	12	12	11	b	70	69	63	59
c	76	29	19	15		260	217	166	61
5 a	81	82	67	46	5 a	81	80	76	70
b	72	14	13	13	b	72	71	62	26
c	80	27	20	20	c	82	211	166	32
6 a	98	100	84	57	6 a	98	90	90	84
b	93	15	14	14	b	93	83	76	59
c	76	29	17	17	c	288	215	182	45
Mean a	84	81	70	48	Mean a	84	80	76	79
b	75	17	12	12	b	75	72	63	46
	86	28	18	18	c	287	220	166	47

— before stability test b — after stability test — blood sugar in mg %

blood donors were incubated at 37 C for 0—6—12 and 24 hours with and without the addition of glucose The results clearly show that when the consumption of glucose continues throughout the experimental period the decrease of the concentration of GSH during the 6-hour periods is almost constant and definitely less than during incubation without the addition of glucose A far better stability of the GSH is also achieved by adding glucose to the blood before incubation (table III)

d) *The pH of the blood during the incubation* In order to evaluate whether the consumption of glucose was determining the rate of decrease of the concentration of GSH of erythrocytes, with or without a test of stability samples of blood from 6 different donors were incubated at 37° C for 0—6—12 and 24 hours with the addition of glucose and either lactic acid or sodium carbonate. The difference of the pH of the blood during incubation created a significant difference in the rate of consumption of glucose. From table IV it is evident that the concentration and stability of the reduced GSH of the erythrocytes was better preserved at the lower pH values.

Table V. The relation between the pH and the temperature during incubation, the time of incubation, and I) the concentration and stability of GSH II) the osmotic fragility of the incubated erythrocytes, and III) the consumption of glucose

	I	II	III
Rising temperature	Decreasing	Decreasing	Increasing
Rising pH	Decreasing	Decreasing	Increasing
Increasing time	Decreasing	Decreasing	Can be kept constant

possible. The effect of menadione sodium bisulfite is known to be inhibition of the reduction of glutathione by a direct inhibition of the glucose-6-phosphate dehydrogenase and the 6-phosphogluconic dehydrogenase (5) and the menadione stability test may thus be a test of the synthesizing and reductive powers of the red cells as regards glutathione. In a previous paper (12) the relation between the osmotic fragility and the pH the temperature, the glucose concentration during incubation and the duration of incubation has been described. Table V summarizes these findings and relates the effect of the factors mentioned to their effect on the concentration and stability of glutathione. As it may be seen from the table a good qualitative correlation exists between the changes of osmotic fragility under the various conditions of incubation and the concentration and stability of gl tathione under the same experimental conditions. The effects of the same factors on the consumption of glucose can also be seen from table IV and it is evident that a simpl relation between glycolysis and the osmotic fragility or the concentration and stability of glutathione cannot exist.

The finding of close correlation between the osmotic resistance of the erythrocytes and the concentration and stability of glutathione is in good accordance with previous investigations

Fegler (6) showed *in vitro* a sharp rise in the percentage of hemolysis of horse blood after exposure to oxygen or after the addition of iodine when the glutathione concentration dropped to about 40 % of its initial value. Benesch and Benesch (2) showed enhanced hemolysis when erythrocytes were incubated with various mercurial compounds assumed to block specifically sulfhydryl groups (2) Jacob and Jandl (9) demonstrated that sulfhydryl inhibition was accompanied by enhanced osmotic fragility and reviewed the pertinent literature. The significance of the relation between the osmotic fragility and the concentration and stability of reduced glutathione remains obscure. The concentration of ATP and the ATP regenerative capacity are closely related to the erythrocyte shape and the active cation-transport capacity (14 16) The active cation transport mechanism has been shown to depend on the activity of an ATP-splitting enzyme system (15) The problem of how glutathione is related to this enzyme or its substrate has not been settled and remains one of the key problems of the biochemistry of the erythrocyte.

Summary

The concentration and stability of glutathione during various conditions of incubation were investigated. The varia

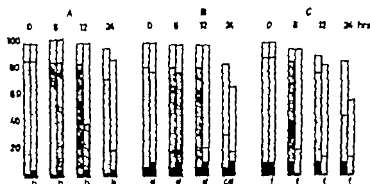


Fig 2 The changes of the concentration and the stability of GSH with time under varied conditions of incubation. A whole column in the figure represents the concentration of GSH. The hatched part of a column represents the concentration of GSH after incubation with menadione. The black part of the columns indicates the average consumption of glucose at the different times. The figure is based on the mean values from table II—III and IV The concentration values were converted to percentage values calculated on the basis of the initial value of the concentration of GSH being = 100 %. In A the temperature was varied a = 5 C., b = 25 C. In B the pH of the blood was varied c = acidified blood, d = alkalinised blood. In C the concentration of glucose was varied e = blood enriched with glucose, f = untreated portion of the same blood sample. The figures above the columns indicate hours of incubation.

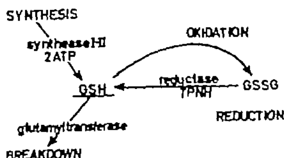


Fig 3 Schematic presentation of the processes that may influence the concentration and stability of GSH and the changes brought about by varying the conditions of incubation.

10 0 or if there was an initial stability test = 13 mg/100 ml erythrocytes with a standard deviation of 1 7

Discussion

The results of the present investigations show a certain relationship between the concentration and stability of reduced glutathione and the consumption of glucose, the temperature, the pH of the incubation medium and the time.

Fig 2 C demonstrates that the maintenance of some consumption of glucose seems essential to the stability and the concentration of glutathione.

Fig 2 A shows, however that at low temperatures and low consumption rates the stability of GSH is definitely better than at higher temperatures and rates of consumption of glucose.

Fig 2 B shows that the stability of glutathione is decreased in alkalinised blood compared to acidified blood in contrast with the consumption of glucose. A tendency for the concentration of glutathione to fall with time after a stability test is evident from fig 2 The concentrations and stability of GSH are the results of several reactions, which shall not be discussed in detail here. It is important to realise however that the level of GSH in the resultant of the processes of synthesis and reduction on one hand and breakdown and oxidation on the other hand and that the effects of temperature, glycolysis and pH are probably exerted via all the processes mentioned (fig 3) The influence of the *in vitro* age is not clear but as shown by Löhr et al several enzymes lose their activity upon storage indeed at different rates (10) thus several explanations of the effect of the duration of storage are

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

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The Development of Diabetes Mellitus During Pituitary Insufficiency

By

Harald M. Frey

Simultaneous occurrence of diabetes mellitus and hypopituitarism, the so-called Houssay phenomenon in man, is a rare finding. Up to 1959 only 21 cases had been reported (3). Since then 9 well documented cases have been added (2, 4, 7, 9, 10, 12) bringing the total up to 30. The cases may be divided into two different groups.

The first group consists of 25 diabetic patients who subsequently developed the syndrome of pituitary insufficiency. Their condition is essentially similar to that of Dr. Houssay's diabetic dogs. *One* interesting feature of these cases is the question whether the diabetic vascular lesions in the pituitary stalk may not be directly responsible for the pituitary infarction in majority of cases, as pointed out by the present author (3). The *other* point of interest is the profound modifying effect on the diabetic state exerted by the pituitary failure. Lately however hundreds of similar cases have been produced surgically through the introduction of hypophysectomy in the treatment of diabetic retinopathy. Thus has been afforded a unique opportunity to study under controlled conditions, the metabolic influence of pituitary failure upon the diabetic state. The importance of the above mentioned cases of spontaneous pituitary failure in diabetics has therefore been reduced in this regard.

The second group is made up of 5 cases (2,9) where the pituitary disease antedates the onset of the diabetes. So far this group has been regarded as little more than a medical curiosity. The extreme rarity of this clinical constellation, however, is no longer the main reason for publishing similar cases: the real importance of these cases is that they may give information on the importance of the pituitary for the development of a diabetic state. The much more so because this condition cannot be reproduced surgically as in group one.

Case report

A 50-year-old housewife was admitted because of anorexia and vomiting of 2 weeks duration. There was no family history of diabetes.

At age 14 she had had an appendicectomy and at age 48 extraction of lenticular cataract on the right eye had been performed.

Submitted for publication October 9, 1963.

tions found were closely correlated to changes of osmotic fragility but not to the consumption of glucose. The significance of these findings is discussed

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in substitution doses. The fact that diabetes mellitus can manifest itself in the presence of long-standing almost complete hypopituitarism seems to contradict the importance of pituitary factors as aetiological agents in this disease.

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sensitivity to small doses of i v insulin was also found, together with hypoglycaemia unresponsiveness. These findings were not altered by substitution doses of cortisone and thyroxin. Finally there was no demonstrable effect of pancreatic beta-cell stimulation

If one accepts the concept of diabetes mellitus being the result of disturbed equilibrium between factors with insulin like effect and factors with diabetogenic or hyperglycaemic effect, the above findings lend themselves to the following interpretation a very extensive lack of pancreatic insulin combined with sharply reduced amounts of diabetogenic factors other than cortisone/thyroxine. This interpretation implies similar effect of diabetogenic factors against endogenous and exogenous insulin which should be a valid assumption in view of the extensive use of exogenous insulin in in vitro experiments on insulin antagonists

There is little doubt that the insulin sensitivity in the present case is due to loss of a pituitary factor That it is not due to the absence of hormones like cortisone or thyroxin has been repeatedly demonstrated clinically both in the present case and in those of Storm Mathisen (11) Kaplan et al (7) and Weller (14) deBodo (1) made similar observations in diabetic, hypophysectomized dogs In all cases, insulin requirement did not increase after physiological substitution doses of the above mentioned hormones.

Much evidence points to the conclusion that the pituitary factor in question is growth hormone. The strong insulin antagonistic and ketogenic effect of human growth hormone has been amply demonstrated in hypophysectomized human diabetics by Luft et al. (8) and Hernberg (6)

The discussion so far has been concerned with the results of the clinical part of this study The in-vitro demonstration of albumin bound insulin antagonistic activity in the patient's serum to a certain extent seems contrary to the clinical results. In the present state of perplexity regarding the complex question of different kinds of insulin antagonists (5) caution is warranted in drawing conclusions from the in vitro results. We know too little to explain what the finding of albumin bound insulin antagonism in the serum really stands for

Vallance-Owen himself however has recently (13) proposed the theory that albumin bound insulin antagonists may be of aetiological importance in diabetes mellitus For these antagonists to exert their action the presence of some functioning pituitary tissue seems to be a condition If this theory holds true, the present case seems to indicate that the amount of functioning pituitary tissue necessary may be very small indeed. The mere fact that diabetes mellitus can manifest itself in the presence of longstanding almost complete hypopituitarism seems to contradict the importance of pituitary factors as aetiological agents in this disease.

The final point of interest is this will our patient eventually develop diabetic retinopathy or is she protected from this ill fate by her pituitary disease?

Summary

Diabetes mellitus developed in a woman of 50 who for over 20 years had been suffering from pituitary insufficiency

Clinical studies gave evidence of very low diabetogenic and ketogenic activity not influenced by cortisone or thyroxine

Acta Medica Scandinavica, Vol. 175, fasc. 5 1964

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Studies on the Osmotic Fragility of Normal Human Erythrocytes

VI. The Concentrations of Glutathione in Fractions of Erythrocyte Populations Obtained by Osmotic Hemolysis

By

Esper Mortensen

The ageing of the normal human erythrocyte is accompanied by a series of changes in the physical and biochemical properties of the cell. The kinetic classification of anemias depends on several rather intricate examinations an important simplification would be achieved if a single parameter expressing mean cell age of a given erythrocyte population could be found. Th concentration of glutathione (GSH) is reported to reflect the activity of the glucose-6-phosphate-dehydrogenase of erythrocytes (2). The activity of thi enzyme varies with the in vivo age of the erythrocytes (3) a decrease of the activity is found as th cell age increases. One would then expect to find a correlation between cell ge nd the concentration of GSH of erythrocytes, and to find a rise of the concentration of GSH of erythrocytes in anemias with increased regeneration and decrease of the concentration in anemias with decreased regeneration. Reports to this effect have been published (1,9)

The present study was designed to measure the concentration of GSH in cell groups of different in vivo age obtained by osmotic hemolysis of normal blood, on the basis of the well-known close correlation between cell age and osmotic fragility (4 8)

Material and methods

Blood from volunteer blood donors was used throughout the investigations. Determination of the concentration of hemoglobin, the erythrocyte count, the leucocyte count, the reticulocyte count, the differential leucocyte count, and the hematocrit was made on all samples by conventional methods.

The determination of reduced glutathione was made by modification of the nitroprusside method previously described (5)

Heparinised blood obtained by venipuncture was slightly packed by centrifugation at 2,000 × g for few minutes and plasma and the buff-colored layer removed. The sample was then thoroughly mixed by inversion and the hematocrit value determined (usually the value was found to be 60 – 70) 3.0 ml of the packed blood were added to 20.0 ml of

Submitted for publication September 30, 1963.

Acta Medica Scandinavica. Vol. 175, fasc. 4, 1964

Book review

Les pancréatites aiguës By Adolphe Bernard 441 p Price 65 F Editions Doin Paris 1963

The author gives a detailed description of the macroscopic and microscopic changes of the pancreatic gland in pancreatitis. The primary change is dislocation and spreading of the lobules due to oedema In the beginning some lobules are normal and others necrotic. In the final stage usually only fragments of cells in necrotic mass are seen and besides, pronounced vasodilatation oedema and haemorrhage. The author gives an account of lesions experimentally produced in rabbits by injecting trypsine. In every tissue of the body the effect is the same necrosis oedema haemorrhage. Collagenase, elastase and lipase also play a rôle in the necrotic processes. In acute pancreatitis there is often a diffusion of pancreatic juice into the retroperitoneal space and into other tissues e. g in the solar plexus. Diffusion also takes place into the blood stream which provokes enzymatic toxaemia

Experimental investigations on the mode of action of the pancreatic enzymes have been performed by the author and are very elucidating He points out that the vasodilatation in acute pancreatitis is due to the effect of histamine which is formed by decarboxylation of histidine and also by direct irritation of the solar plexus. The effect of lipase occurs later than the necrose provoked by trypsine. The great importance of trauma is discussed for instance in connection with surgical intervention in the abdomen. The various factors provoking acute pancreatitis have one thing in common they give rise to diffusion of pancreatic juice from the acini into the surrounding connective tissue, where activation of trypsine takes place

The symptoms of acute pancreatitis and their genesis are described in about 200 pages The author gives an excellent description of the "pancreatic drama its symptoms and prognosis. He reports on different kinds of treatment and among others stresses the importance of antishock treatment in the first stage of the condition

The pathogenesis and differential diagnosis of acute pancreatitis are still far from clear The book gives a very interesting and instructive description of some new findings of great importance for the understanding of this abdominal disease, which often in spite of new methods, is difficult to recognize

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3.0 ml of blood initially added, as calculated from separate determination of the concentration of GSH of the blood samples.

The details of the calculation procedure are omitted here but the data necessary for the calculation are presented in table I the concentration in the tw fractions as µg GSH/ml of the final reaction mixture, which is 7 ml, the hematocrit value of the blood after the packing, and the percentage of hemolysis. A calculation of the concentration of the non-hemolysed cells can be done on the basis of the same figures, but was not performed here.

Results

The result of the present investigations appear from table II. Twelve normal samples of blood were fractionated by osmotic hemolysis. The concentrations determined on the fractions were all very close to those determined on the unfra tionated blood samples. The results of the control measurements and calculations appear in table II. Relative to the concentration of GSH in whole blood the recovery in the fractions was 94—109 %. Thus the experimental data indicate that the concentration of reduced glutathione is equal throughout a given normal erythrocyt population. If any difference be tween the concentrations of GSH of young and old red cells does exist it is so small that t ca not be demonstrated with the present experimental technique.

Discussion

Only few data on the relation between the concentration of glutathione and or u cell age exist. Prankerd (7) 1958 was unable to demonstrate any significant differenc in the concentration of GSH between the top and bottom layers after centrifugation of 3 normal samples of blood.

Table II. The amount of GSH in the various fractions of the hemolytic mixtures compared with the total amount of GSH added. The values of GSH are µg

No.	Amount of GSH				
	I	II	III	I + II	
	I remainder	I hemolysate removed	Added as whole blood		Recovery (%)
1	160	280	431	440	102
b	199	232	431	432	100
	306	127	431	433	100
2	138	246	378	384	102
b	176	180	378	356	94
	312	59	378	371	98
3	190	365	561	556	99
b	209	320	561	529	94
	434	145	561	579	103
4	201	367	564	568	100
b	277	294	564	571	101
	513	101	564	614	109
5	129	247	378	375	99
b	140	230	378	370	98
	277	104	378	381	101
6	141	268	410	409	100
b	157	257	410	414	101
	267	168	410	435	106
7	159	297	456	456	100
b	206	240	456	446	98
	334	133	456	467	102
8	141	264	406	405	100
b	186	228	406	414	102
	393	57	406	440	108
9	122	219	336	341	102
b	153	181	336	334	99
	300	49	336	349	104
10	138	259	398	397	100
b	188	206	398	394	98
	352	49	398	401	101
11	139	253	388	392	101
b	156	238	388	394	102
	300	108	388	408	105
12	132	251	385	383	99
b	174	197	385	371	96
	342	48	385	388	101

Table I. The concentration of GSH as measured on the various fractions

No.	Hematocrit	Hemolysis (%)	Conc. of GSH in		
			Hemolysate in µg/ml of the final solut.	Remainder in µg/ml of the final solut.	Hemolysate as mg/100 ml cells
1 a	58	100	17.2	8.3	74
b		91	14.2	10.3	67
c		42	7.8	15.8	73
2 a	54	100	15.1	7.1	70
b		79	11.0	9.1	63
c		23	3.6	16.1	64
3 a	75	100	22.4	9.8	75
b		83	19.6	10.8	78
c		31	8.9	22.4	87
4 a	71	100	22.5	10.4	79
b		77	18.0	14.3	79
c		21	6.2	26.5	93
5 a	73	100	15.1	6.6	52
b		84	14.1	7.2	57
c		32	6.4	14.3	62
6 a	73	100	16.4	7.3	56
b		88	15.8	8.1	61
c		46	10.5	13.8	71
7 a	77	100	18.2	8.[illegible]	59
b		76	14.7	10.6	61
c		41	8.2	17.2	59
8 a	77	100	16.2	7.3	53
b		83	14.0	9.6	54
c		15	3.5	20.3	66
9 a	65	100	13.4	6.3	5[illegible]
b		77	11.1	7.9	54
c		17	3.0	15.5	62
10 a	73	100	15.9	7.1	55
b		75	12.6	9	56
c		17	3.0	18.[illegible]	54
11 a	66	100	15.3	7.[illegible]	59
b		87	14.6	8.1	61
c		33	6.6	15.5	64
12 a	63	100	15.4	6.8	59
b		77	12.1	9.0	59
c		14	2.8	17.7	69

hemolytic solutions chosen to give 100 and approximately 80 and 20 of hemolysis. The hemolytic mixtures were allowed to stand for 15 minutes at room temperature and then centrifuged at 3,000 × g for 4–5 minutes. After centrifugation 3 × 5.0 ml of the supernatant fluid were pipetted into 3 centrifuge tubes 2 for the determination of GSH and one for the determination of the filtrate blank value. The degree of hemolysis was determined by pipetting 50 µl of the supernatant solution into 5.0 ml of Drabkin solution and reading the optical density at 525 nm. The percentages of hemolysis were calculated by dividing the optical density of the solutions by the density of the solution yielding 100 hemolysis and multiplying this ratio by 100. The hemolytic solutions were prepared as previously described (6). The hemolysates were precipitated at 37° C with 2.0 ml of 13 sulfosalicylic acid and were allowed to stand for 10 minutes at 37° C before filtration through Schleicher & Schüll filtering paper no. 589. 3.0 ml of the clear colorless filtrate were pipetted into centrifuge tubes and placed in a water bath at 10 C. The development of the color with nitroprusside and the reading of the samples, standards, and blanks closely followed the procedure previously described.

To the remaining contents of the tubes with the hemolytic mixtures 10.0 ml of distilled water were added, and the tubes were allowed to stand at room temperature till complete hemolysis was obtained (about 15 minutes). 3 × 5.0 ml of these hemolysates were pipetted into centrifuge tubes and determination of the concentration of GSH in duplicate and of the filtrate blank was performed as described above.

Calculation

On the basis of the concentrations of glutathione obtained (in µg/ml of the final reaction mixture) a calculation of the concentration of GSH in the different fractions could be carried out. The results were expressed as mg GSH/100 ml erythrocytes.

As a check on the results obtained the amount of GSH removed from the hemolytic mixture with the hemolysate and the amount of GSH of the remainder were calculated. If the analysis had been correctly performed the sum of the amount of GSH of these fractions should be equal to the amount of GSH of the

Acta Medica Scandinavica. Vol. 175, fasc. 5 1964

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Splenectomy in Myelofibrosis

By

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Few diseases offer less opportunities for therapeutic generalizations than myelofibrosis. The range of problems presented extends from severe erythrocytosis to pronounced anaemia, from marked thrombocytosis to extreme thrombocytopenia. The therapeutic problems are often found to change with the progress of the disease in the individual patient. The anaemia and thrombocytopenia present the greatest difficulties of pathogenetic interpretation, with the aim of rational therapy. In stages which present pronounced anaemia and thrombocytopenia, the spleen is almost always markedly enlarged, not uncommonly to an extreme degree. It seems quite reasonable, therefore, that the sign of splenomegaly has acquired dominant position in the discussion on the pathogenesis of myelofibrosis. The pathogenesis, however remains obscure. The two most favoured theories may be summarized as follows.

1. In myelofibrosis the primary change is the replacement of the haemopoietic elements of the bone marrow by fibrous tissue, reticulum cells or bony tissue. A secondary change, compensating for the haemopoietic tissue which has been supplanted from the bone marrow is the development of myeloid metaplasia, in the spleen in the first instance, but also in the liver, lymph nodes and other organs (11, 19, 33).

2. Myelofibrosis, together with chronic myeloid leukaemia and polycythaemia, constitute a myeloproliferative state. This is an indication of the multipotential properties of the primitive mesenchymal cell, and in the case of myelofibrosis represents increased differentiation to fibroblasts, osteoblasts or reticulum cells, with the resultant conditions of fibrosis, sclerosis or reticulosis of the bone marrow. These changes are found not only in the bone marrow but also in other organs, so that the spleen, liver and lymph nodes become the seat of changes concurrent with those of the bone marrow. At the same time, an increased differentiation into haemopoietic elements is seen in certain phases of the disease, leading to myeloid metaplasia and possibly erythrocytosis, leucocytosis and/or thrombocytosis (6, 7, 10, 14, 16, 29).

It is beyond the scope of this article to discuss in further detail these theories of the pathogenesis of myelofibrosis, but

Submitted for publication September 19, 1963.

These findings are in contrast to previous reports (1 9) of increasing concentrations of GSH/100 *ml* erythrocytes in patients with rapid regeneration of erythrocytes after hemorrhage, where a decreased mean age of the erythrocytes may be anticipated Of interest in this connection is the demonstration by Tomita (9) of a significant inverse correlation between the values for the concentration of GSH/100 ml erythrocytes and the haematocrit in patients with various anemias and in *normal subjects* This might imply that a systematic error of analysis influences the results Tomita (10) demonstrates in another paper that a dilution of the samples before deproteinization results in a fall of the concentration values for a given sample with increasing dilution In a previous paper (5) the author demonstrated that the concentration of GSH in a given sample of blood was inversely correlated to the concentration of acid used for the precipitation of the proteins. The acid binding capacity of the blood proteins will thus influence the results obtained Consequently the higher the hematocrit value of a given sample the lower will be the concentration of GSH found. A discussion of this effect of the protein concentration is outside the scope of this paper however it seems rational to correct the hematocrit values to a fixed level before determining the concentration of GSH of different samples for comparative purposes.

Summary

The concentration of glutathione of groups of red cells of different *in vivo* age obtained by osmotic hemolysis of blood from 12 normal blood donors has been investigated No significant variation of the concentration of glutathione with cell age was found

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were found all over the skin. The spleen reached the iliac crest, the liver was not palpable, there were no palpable lymph glands. Hb 13.3 g%, R.B.C. 6.5 mill./µl, W.B.C. 13,200/µl with normal distribution. The erythrocyte picture showed poikilocytosis and anisocytosis. There were no normoblasts in the peripheral blood. Thrombocytes 26,000 µl. Coombs test negative. Bleeding time: 20 min. Coagulation time 3 1/2 min. Prothrombin 100 %. Icterus index 10. Basal metabolic rat 144 %. ESR 23 mm, ECG left-sided axis deviation. Roentgenographic studies of the long bones showed nothing abnormal. Sternal puncture was not performed in view of the bleeding tendency

It was considered that surgery was indicated on the basis of the severe thrombocytopenia with pronounced haemorrhagic diathesis. Splenectomy was performed on the 12th August 1952 (Surgical Department C) The spleen weighed 1 700 g and measured 22 × 14 × 8 cm. Microscopy showed pronounced extramedullary haemopoiesis. The post-operative course was uneventful. After splenectomy the following laboratory values were obtained Hb 14.7 g% R.B.C. 2.79 mill/µl. Leucocytes 50,000/µl with 5 % immature cells. N normoblasts in the peripheral blood. Thrombocytes 49,000/µl, rising rapidly to 100,000/µl. The haemorrhagic diathesis ceased following the splenectomy B.M.R. 125

Fiv months after the splenectomy the patient was readmitted to the department with angina pectoris. She had been in good health in the meantime. There was no sign of haemorrhagic diathesis, and no hepatomegaly Hb 12.7 g% R.B.C 4.32 mill /µl. Leucocytes 25,000/µl with normal distribution. No normoblasts in the peripheral blood. Thrombocytes 149,000/µl.

The patient was admitted to hospital again for her angina pectoris, 2 1/2 years after the splenectomy She had meanwhile been in good health. A number of ecchymoses were observed on the trunk. The liver was not enlarged Hb 8.0 g% R.B.C. 2.77 mill./µl. Leucocytes 32,000 µl with 1 immature cells There were 16 normoblasts per 100 nucleated cells in the peripheral blood. Thrombocytes 114,000 µl. Bleeding time 4 min Coagulation time 3 min. Capillary resistance was much reduced. The patient received transfusions until the Hb-concentration reached 10.3 g%. Sternal puncture gave a "dry tap"

The patient was once again admitted just over four years after the splenectomy There was moderate angina pectoris with dyspnoea and fatigue. There were numerous ecchymoses all over the skin. The liver was not palpable below the costal margin. Hb 3.9 g% R.B.C. 1 79 mill./µl. Leucocytes 22,000 µl with 7 % immature cells in the peripheral blood. Thrombocytes 83,000–153,000/µl. B.M.R. 150 %. A tumour was found in the left breast. The patient received transfusions and was transferred to Surgical Department R, where the left breast was amputated. Microscopy showed carcinoma colloides.

Thereafter the patient was not seen again in the department. In July 1958 – 6 years after splenectomy – the patient was admitted to Department II The Municipal Hospital, Copenhagen, with haematemesis, melaena and jaundice. The liver could be palpated four fingerbreadths below the costal margin. The patient died soon after admission. The only laboratory study performed was Hb 11.3 g %.

At autopsy (Dr K. E. Sjølin, M. D Institute of Pathology University of Copenhagen) the bone marrow showed many collagen fibrils and fibroblasts, together with fair number of megacaryocytes, myelocytes and erythroblasts. There was no sign of leukaemia. Diagnosis myelofibrosis. Extramedullary haemopoiesis in liver and lymph nodes. Cirrhosis of the liver

Comments

This may ha e been a case of poly cythaemia originally which progressed into myelofibrosis. The indication for splenectomy was thrombocytopenia with pronounced tendency to haemorrhage. Following splenectomy the thrombocyte figure rose rapidly to almost normal levels, and remained there, but this did not hinder the development of non thrombocytopenic haemorrhagic diathesis 4 years after the splenectomy although not in as pronounced a degree as previously The patient had developed

most of the observations available are decisively in favour of the second theory (2 6 13 21 25 26 28)

No grounds exist therefore, for allowing theoretical considerations on the pathogenesis of myelofibrosis to play any role in the evaluation of possible indications for splenectomy Here as elsewhere, clinical experience should be the basic guide. The first reports on splenectomy in myelofibrosis were by no means encouraging Hickling (15) in 1937 collected from the literature a total of 27 patients with myelofibrosis, in whom splenectomy had been carried out Of these, 15 died immediately post-operatively or within the first four weeks after operation and of the remaining 12 six had died within one year A series of reports describing correspondingly poor experience with splenectomy in myelofibrosis appeared during the next few years, all recording considerable post operative mortality (5 8 12 14 20 23 27 33)

In more recent years, however rather more light and shade have been introduced into this picture The new point of view which has been stressed is that splenectomy when performed on certain indications, may have a beneficial effect (1 2 4 19 25 31) For example, Green et al. (13) in 1953 collected 29 patients whose case history had been published since Hickling's 1937 report (15) Only 6 of these 29 patients had died within the first six months after the splenectomy Seventeen survived the intervention for two years and 9 for four years. From this analysis of the material, no definite argument could be presented to indicate that loss of the spleen in myelofibrosis was harmful In 8 patients, a dramatic haematological improvement was observed following splenectomy

Bouroncle and Doan (6) have recently published their results with splenectomy in myelofibrosis, covering a period of 16 years Good results were obtained in 14 out of 24 subjects who underwent the operation. Following splenectomy 9 patients did not require transfusions, and in five the requirements for transfusion were reduced considerably These favourable results lasted from 6 months to 4 years. Only two of the patients died within the first two days after operation.

The operative mortality is thus no longer prohibitive, so that discussion as to whether splenectomy is indicated in certain cases of myelofibrosis has once more become topical The most important problems may be briefly stated as follows

A. What is the operative mortality

B. What are the indications for the operation

C. To how great an extent does splenectomy improve the blood status of the patient.

D Is there any risk of splenectomy being followed by deterioration of the patient's haematological status.

During the period 1952–1962 a total of 25 patients with myelofibrosis was admitted to Medical Department A, Rigshospitalet. Six of these patients presented indications for splenectomy The purpose of this communication is to report the results in these patients. The material is numerically modest, but as no really extensive series have been published a report of these six cases seems justified especially for the benefit of subsequent reviews.

Case reports

Case 1 (previously published (31)) A 61 year-old dressmaker's fitter admitted with a history of haemorrhagic diathesis and angina pectoris for five years. Palm-sized suggilations

150,000/μl with 29 immature cells. There were up to 48 normoblasts per 100 nucleated cells. Thrombocytes 148,000/μl. B.M.R. 179 %. Roentgenography of the spinal column and pelvis showed pronounced sclerosis. The patient was given Mitostan® (busulfan) in an attempt to lower the B.M.R. and reduce the hepatomegaly. After treatment for five weeks, however the B.M.R. and hepatomegaly remained unchanged. In spite of repeated transfusions the Hb value fell to 8.6 g%. Leucocytes 110 000 μl. Thrombocytes 260 000/μl. The patient's condition deteriorated rapidly and he died 14 months after the splenectomy in cachexia.

At autopsy (Dr. Emmerik Jensen, M. D. Institute of Pathology, University of Copenhagen) the bone marrow was found to be replaced by fibrous tissue. There was scanty erythropoiesis and myelopoiesis. The liver weighed 6.5 kg. There was considerable extramedullary haemopoiesis in the liver and lymph glands. No leukaemic infiltrates. No signs of cirrhosis of the liver. Diagnosis myelofibrosis.

Comments

Splenectomy was performed in this patient on the following indications: 1. Pronounced anaemia. 2. Thrombocytopenia. 3. Discomfort from the very large spleen. 4. Pronounced hypermetabolism. Cautious irradiation had a good effect on both anaemia and thrombocytopenia, and the spleen decreased in size so that there was reason to believe that splenectomy would improve the patient's condition. This expectation was fulfilled as following splenectomy his condition was considerably better during the first 8 months. Hb lay at a higher level and was almost normal at times, while the thrombocyte figure was normalized. However gradual enlargement of the liver ensued, with portal stasis and hypermetabolism, and the patient died in cachexia.

Case 3. 54-year-old furniture remover who had suffered from fatigue during the past two years, transferred to this department from Department III, Copenhagen Municipal Hospital. An Hb value of 6.1 g% was found. After several negative attempts, a marrow sample was obtained by iliac crest biopsy. This showed signs of myelofibrosis. The patient had been given about 25 transfusions in the course of the past 18 months.

The patient was obviously anaemic. The liver reached 1 fingerbreadth and the spleen 4 fingerbreadths below the costal margin. There was some ascites and moderate oedema of the ankles. Hb 7.0 g%. R.B.C. 1.77 mill./μl. Reticulocytes 38 ‰. Leucocytes 1,500/μl with 2 % immature cells and relative lymphocytosis. The erythrocyte picture showed pronounced anisocytosis and poikilocytosis. There were no normoblasts in the peripheral blood. Thrombocytes 18,000/μl. Haptoglobin 152 mg%. Bleeding time 3 min. Coagulation time 3 min. E.S.R. 28 mm. Serum bilirubin 1.2 mg%. Thymol turbidity 0.04 (normally < 0.15). Alkaline phosphatases 47 units/100 ml. GP transaminases 14 units/ml (normally < 15). Bromsulphalein retention 11.2. Serum albumin was considerably reduced 2.7 g%. Coombs test negative. Sternal puncture was attempted. The bone felt very hard and only peripheral blood was aspirated. In spite of the reduced liver function, splenectomy was decided upon, as the patient still required frequent transfusions, and tagging of his erythrocytes with radioactive chromium showed them to have a considerably reduced life time. At the same time he had considerable thrombocytopenia, although without haemorrhagic diathesis.

Splenectomy was performed in Surgical Department D on the 2nd March 1960. There were 3 l ascites. The spleen was the size of an ostrich egg, measuring 17 × 14 × 6 cm. Histopathological examination showed splenic fibrosis with deposits of iron-containing pigment. The liver was somewhat enlarged, the cut surface showing a slight nutmeg appearance. Liver biopsy showed slight interstitial fibrosis and non-specific inflammation. No definite extramedullary haemopoiesis was found on microscopy of spleen and liver. After the operation the Hb level at first remained constant at 12–14 g%. R.B.C. about 4 mill./μl. Leucocytes about 2,000 μl with slight shift to the left. There were no normoblasts in the peripheral blood. The thrombo-

anaemia 2 1/2 years after the splenectomy but nevertheless lived for a further six years without excessive requirements for transfusion. The leucocyte count rose steadily following splenectomy and some shift to the left developed. The hepatomegaly did not develop until terminally As far as could be judged the cause of death was liver cirrhosis perhaps as an aspect of the myelofibrosis

Case 2 A 48-year-old male confidential clerk was admitted to hospital with a six months history of frequent attacks of pain in the region of the spleen and increasing abdominal circumference. The patient also complained of fatigue, loss of weight, increased tendency to sweat, exertional dyspnoea and slight periodic rise of temperature The patient was found to be pale, almost cachetic. The abdomen was prominent with the veins readily visible. The spleen almost reached the midline and was three fingerbreadths from the umbilicus. The liver was not palpated below the costal margin There was no ascites, swollen lymph glands or haemorrhagic diathesis. Hb 8.0 g%. R.B.C. 2.57 mill./μl. Leucocytes 10,300/μl with 9 % immature cells. No normoblasts in the peripheral blood. The erythrocyte picture showed very pronounced aniso-poikilocytosis and some ovalocytosis. Thrombocytes 119 000/μl. Reticulocytes 9—39 /‰ E.S.R. 14 mm. Coombs test negative Bleeding time 2 min. Coagulation time 3 1/2 min. Icterus index 6 B.M R. 148 %. Sternal puncture was attempted on three occasions, but nothing could be aspirated. The bone felt very hard A costal biopsy was therefore carried out, showing marrow tissue with reticulosis and cell polymorphy presumably an early stage of myelofibrosis.

During his admission the Hb showed a slightly falling tendency and thrombocyte level fell to 45,000/μl. Splenic irradiation therapy was then tried, a dose of 125 r being given, with 75 r to the upper half and 50 r to the lower half of the spleen. As a result the pain in the splenic region decreased considerably reticulocytosis (128 /‰) developed and a rise in Hb the thrombocyte figure rose to 100,000/μl. As the irradiation therapy had had a good effect but caused the patient considerable discomfort, it was decided to perform splenectomy which was carried out in Surgical Department C on 27th January 1956. The spleen weighed 4 6 kg and measured 48 × 37 × 28 cm. Microscopy of spleen and liver (biopsy taken during operation) showed extramedullary haemopoiesis. The post-operative course was uncomplicated. Hb after operation reached 13.0 g% (after numerous transfusions) In the course of three weeks the Hb fell to 10 4 g% and remained at this level. Reticulocytes 8 /‰ R.B.C. 3.58 mill./μl. Leucocytes 6,800/μl with 17 % immature cells. The erythrocyte picture showed pronounced aniso-poikilocytosis. There were no normoblasts in the peripheral blood. Thrombocytes 259 000/μl. B.M.R. 122 The patient felt considerably better than before the operation.

The patient was seen again five months after the operation There was now increasing abdominal circumference. The liver extended to a couple of fingerbreadths below the umbilicus. Hb 10.8 g% R B.C. 3 6 mill./μl. Leucocytes 44 000/μl with shift to the left. There were 14 normoblasts per 100 nucleated cells in the peripheral blood. Thrombocytes 400 000/μl. In spite of the hepatomegaly there was no sign of reduced liver function, apart from low prothrombin value (50 %) In view of the patient s clinical condition, for example his hypermetabolism (B.M.R. 154) treatment with prednisone was instituted, 7 1/2 mg daily whereupon his general condition improved.

The patient was still suffering from fatigue and a tendency to sweating 8 months after the splenectomy but was nevertheless able to go on working four hours daily Hb 14 4 g% R.B.C. 5 17 mill./μl Leucocytes 83,000/μl with 25 immature cells. There were no normoblasts in the peripheral blood. Thrombocytes 308,000/μl.

In January 1957 — one year after splenectomy — the patient was admitted with pronounced discomfort in the form of fatigue tendency to sweating, dyspnoea on exertion and abdominal tension. The liver was found enormously enlarged, filling the entire right half of the abdomen to the symphysis. There was some ascites, congestion of the veins o the neck, tortuosity of the abdominal eins and oedema of the lower limbs. Hb 12.7 g%. R.B.C. 6.0 mill./μl. Leucocytes 100,000—

with 85 % immature cells, including promyelocytes, and only 8 % granulocytes. There were 72 normoblasts per 100 nucleated cells in the peripheral blood. The patient's condition was satisfactory following splenectomy and she was discharged from hospital five weeks after operation.

Two months after operation she was admitted to the Medical Department of Aalborg Municipal Hospital suffering from bronchopneumonia, and died in spite of antibiotic treatment. On admission Hb was 6.9 g%. R.B.C. 2.74 mill./µl. Leucocytes 11 000/µl, with pronounced shift to the left as previously and few granulocytes (10 %). There were 64 normoblasts per 100 nucleated cells.

At autopsy (Dr B. Jacobsen, Pathological Institute Aalborg City and County) bronchopneumonia was found. Histopathological examination showed fibrous conversion of the marrow with increased content of collagen fibrils and reticulum cells. Considerable extramedullary haemopoiesis was found in the liver kidneys, adrenals and retroperitoneal lymph glands.

Comments

In this patient, the indications for splenectomy were as follows 1 Severe haemolysis, demonstrated by the considerable transfusion requirements and the greatly reduced erythrocyte life-time Surface measurements over the spleen after tagging the patient erythrocytes with Cr^{51} demonstrated the probability that the spleen was of decisive importance for the haemolysis 2. Thrombocytopenia. This remained unaffected, while haemolysis decreased, so that Hb-concentration was stationary 3 weeks after operation. When death occurred two months after splenectomy the Hb-concentration had fallen somewhat. Considerable normoblastosis developed in the peripheral blood Death was due to intercurrent infection. Nevertheless, reduced resistance to infection on account of anaemia and prednisone treatment, together with granulocytopenia presumably contributed to the fatal course of the bronchopneumonia.

Case 5. 58-year-old cabinet maker previously admitted to the psychiatric department on account of abuse of alcohol, character deviation, and narcolepsy Treated with digitalis for two years because of auricular fibrillation. The patient had never had rheumatic fever During the last ten months the patient had experienced considerable discomfort from his enlarged spleen. Pronounced splenomegaly was found during admission to Department III of the Copenhagen Municipal Hospital. Hb 14.8 g% Leucocytes 3,900/µl with some lymphocytosis. Thrombocytes 230,000 µl. Biopsy of the iliac crest showed incipient fibrosis of the marrow of the bone, presumably an early stage of myelofibrosis. During the next few months the patient suffered considerable discomfort from his swollen spleen, with frequent violent pain localized to the region of the spleen. He also suffered from attacks of sweating, loss of weight, pruritus, and cutaneous haemorrhages. The patient was readmitted to the same department, where Hb was now found to be 7.3 g%. Leucocytes 1,000/µl. Thrombocytes 17 000/µl. The patient was then transferred to Medical Department A, Rigshospitalet, for a decision as to splenectomy

The patient was very pale. The spleen reached the umbilicus. The liver was felt two fingerbreadths below the costal arch. No cutaneous haemorrhages or other haemorrhagic diathesis. Hb 4.8 g%. R.B.C. 1.6 mill./µl. Leucocytes 550–1,500/µl (60 of these lymphocytes) No immature cells. There were no normoblasts in the peripheral circulation. The erythrocyte picture showed anisocytosis. Thrombocytes 13,000/µl. Coagulation time 5 1/2 min. Bleeding time 4 1/2 min. Coombs' test negative. Serum iron 236 µg% Transferrin 319 µg% Reticulocytes 6–28 /... E.S.R. 20 mm/h Haptoglobin 0–7 mg% Serum bilirubin 0.9 mg%. Prothrombin 140 % ECG auricular fibrillation. Cr^{51}-marked erythrocytes showed half-life of 18 days in contrast to normal value of about 25 days, indicating quite considerable haemolysis. In view of the haemolytic anaemia and the pronounced thrombocytopenia, treatment with

cyte count did not increase significantly after splenectomy lying around 35 000–40 000/μl. There was still no haemorrhagic diathesis. A left-sided subphrenic abscess developed however with considerable rise in temperature, and Hb started to fall. Laparotomy was performed, with resection of the IX rib and evacuation of the subphrenic abscess, but the patient's condition deteriorated and he died of cardiac and hepatic failure about two months after splenectomy.

Autopsy (Dr E. Badsberg Institute of Pathology University of Copenhagen) showed myelofibrosis and pronounced extramedullary haemopoiesis of liver and lymph nodes. Histopathological examination of the bone marrow showed the medullary cavities to be filled with reticular tissue, which contained scattered lymphocytes and plasma cells. A few small islands of haemopoietic tissue were seen here and there. Small patches of calcification were also seen in the medullary spaces.

Comments

Splenectomy was found to be indicated because of haemolysis with a considerable need for transfusions. Correspondingly operation appears to have brought the haemolysis under control. Splenectomy had no effect on the thrombocytopenia. The course of the disease was complicated however by a subphrenic abscess. This complication together with the poor liver function contributed significantly to the fatal outcome.

Case 4 Seventy-year-old widow who suffered from severe anaemia already seven years before admission. At that time, during admission to Aalborg Municipal Hospital, repeated attempts were made to perform sternal puncture, but nothing could be aspirated. Bone biopsy showed very cell-poor marrow with considerable fibrosis, presumably myelofibrosis. At that time there was no splenomegaly. The patient received numerous blood transfusions. During the next seven years she managed without transfusions. The patient was then readmitted to the same department, this time with the diagnosis of acute abdomen. The spleen was found enlarged. Hb 8.4 g%. Leucocytes 8,800/μl with some shift to the left. Bone biopsy once more showed a very cell-poor marrow with considerable fibrosis. An attempt was made to raise the patient's haemoglobin level by means of transfusions, but a temperature reaction developed whereupon the patient was transferred to Medical Department A, Rigshospitalet, for a decision as to splenectomy.

On admission the patient was very pale. The spleen reached one handsbreadth below the costal margin and the liver two finger breadths. There were remains of older cutaneous haemorrhages, but no recent ones. There was no ascites. Hb 6.1–4.1 g%. Erythrocytes 2.78 mill./μl. Reticulocytes 25 /‰. Leucocytes 5 500/μl with 24 immature cells. No normoblasts in the peripheral blood. The erythrocyte picture showed considerable aniso-poikilocytosis. Thrombocytes 41 000/μl. Serum iron 128 μg%. Transferrin 196 μg%. Haptoglobin 27 mg%. Serum bilirubin 0.5 mg%. B.M.R. 118 %. Iliac crest biopsy showed very cell-poor marrow with 70 normoblasts. Tagging the patient's erythrocytes with Cr^{51} showed that their lifetime was considerably reduced, the half-life being 12 days, compared with a normal 25 days. External measurements over the spleen and liver showed increased activity over the spleen with a spleen/liver ratio rising from 3.6 to 6.7 in the course of 11 days, suggesting increased erythrocyte destruction in the spleen.

In spite of numerous blood transfusions the Hb fell rapidly so the patient was given large doses of prednisone. This reduced the transfusion requirements somewhat, but there was still difficulty in keeping the Hb concentration at a reasonable level. In view of the considerable transfusion requirements and the pronounced thrombocytopenia (4 800/μl) splenectomy was decided on.

This was carried out on 1st February 1961 in Surgical Department D. The spleen measured 25 × 18 × 13 cm. Microscopy showed fibrosis and extramedullary haemopoiesis. The postoperative course was uneventful. After splenectomy treatment was continued with prednison 20 mg daily. Hb remained stationary around 10 g% while the thrombocytopenia persisted (28 000–10,000/μl) but without haemorrhagic diathesis. Leucocytes 7,500/μl

Table I.

Pat. no	Indications for splenectomy	Result of splenectomy		Died after splenectomy
		Hb concentration	Thrombocytes	
1	Thrombocytopenia	Normal before op.	++	—
2	Haemolytic anaemia, thrombocytopenia	+ to ++	++	—
3	Haemolytic anaemia, thrombocytopenia	+	(+)	+
4	Haemolytic anaemia, thrombocytopenia	+	—	—
5	Haemolytic anaemia, thrombocytopenia	++	++	—
6	Haemolytic anaemia, thrombocytopenia	++	++	—

— no, (+) slight, + definite, ++ marked improvement.

The patient felt considerably better after the operation. However there was quit pronounced and persistent gastro-intestinal haemorrhage. In spite of intensive studies (repeated roentgenography of the gastro-intestinal canal, endoscopy and string-test) the aetiology of the haemorrhage could not be found. Despite the haemorrhage, the Hb concentration remained constant at 9–10 g%. Leucocytes rose to 20,000–25,000/μl, still with considerable shift to the left (up to 11 % immature cells). There were about 75 normoblasts per 100 nucleated cells in the peripheral blood. Platelets rose to 196,000/μl (immediately prior to operation they were 11,000/μl). Reticulocytes 30–100 ‰. E.S.R. 21 mm/h. B.M.R. fell to 116. There was now no haemorrhagic diathesis. Cr^{51} measurement was repeated and the half-life value of 17 days obtained, still reduced but now considerably less than before splenectomy, when it was 8 1/2 days. It was not until about 2 1/2 months after splenectomy that the Hb began falling because of loss of blood from the intestinal canal. As the aetiology of this could not be found, exploratory laparotomy was performed three months after splenectomy. A duodenal ulcer was found and laparotomy with Billroth I gastrectomy and transabdominal vagotomy was performed. There was diffuse oozing of blood post-operatively, the patient collapsed and death ensued.

At autopsy (Dr. Werdelin, Institute of Pathology, Copenhagen University) pronounced hepatomegaly and haemoperitoneum were found. Histopathology of the spinal column and marrow of the femur showed severe pathological changes, the marrow being replaced by loose connective tissue with no sign of myelopoesis in most sections. A number of reticulum cells were seen. There was nevertheless the impression that in places there was haemopoiesis. A microscopical examination of the liver showed signs of acute congestion. The lymph nodes showed pronounced sinus reticulosis and myeloid metaplasia.

Comments

Indications for splenectomy were found on the following grounds: 1. Severe haemolysis (considerably reduced erythrocyte survival time by Cr^{51} tagging); from the surface measurements the spleen had to be considered a significant factor in the haemolysis. 2. Thrombocytopenia with haemorrhagic diathesis. 3. Hypermetabolism. 4. Severe hyperuricaemia with nephrolithiasis. After splenectomy the Hb remained at a considerably higher level for 2 1/2 months, after which bleeding anaemia developed. The platelet count became normal following the splenectomy and the haemorrhagic d

prednisone was started 40 mg daily for one week and then 20 mg daily for one week. As no effect on the anaemia or thrombocytopenia was observed, however the prednisone was slowly withdrawn It was then decided to perform splenectomy

Operation was carried out in Surgical Department C on 22nd June 1961 after the patient had received blood transfusions. The spleen measured 25 × 16 × 10 cm. Microscopy showed hyperplasia of the reticulum cells. The histopathological lesions were consistent with the diagnosis of myelofibrosis. The postoperative course was uneventful. The patient's condition was satisfactory after splenectomy and he was soon discharged from hospital. Hb 12 3 g% Leucocytes 4,200/μl with some lymphocytosis (60 % lymphocytes) No immature white cells or normoblasts in the peripheral blood. The erythrocyte picture showed anisocytosis. Platelet count 124 000/μl Haptoglobin 206 mg% E.S.R 13 mm/h

Since discharge, the patient has been admitted at times to a psychiatric department (Department E, Sct. Hans Hospital) for abuse of alcohol. After the splenectomy the patient has been completely well somatically and the haematological values have become normal. As of March 1963 — 21 months after the splenectomy — the patient still feels well Hb 14 9 g% Leucocytes 6,500/μl with only slight lymphocytosis, moderate shift to the left, and a few normoblasts in the peripheral blood Erythrocyte picture shows anisocytosis. Platelet count approx. 200 000/μl. No haemorrhagic diathesis

Comments

Splenectomy was performed on the following indications 1 Severe anaemia with a considerable haemolytic component continuous need for transfusion reduced erythrocyte life time very low haptoglobin and slight reticulocytosis. 2 Pronounced thrombocytopenia 3 Severe leucopenia, particularly neutropenia (leucocyte counts as low as 500/μl) 4 Rather pronounced discomfort from the enlarged spleen Nearly two years after the splenectomy the patient feels completely well. Hb-concentration is normal Platelet count is normal there is no haemorrhagic diathesis. Leucocyte count is also normal, with only slight shift to the left

Case 6. 65-year-old widow admitted with a history of symptoms of anaemia of four years duration. In the last six months cutaneous haemorrhages after slight trauma, and painful sense of pressure in the abdomen. The spleen reached the midline and three cm below the umbilicus. The liver reached 4 fingerbreadths below the costal margin. No enlargement of lymph nodes. A number of suggillations were seen on the extremities. Hb 6.5 g%. Erythrocytes 2.07 mill./μl Reticulocytes 9 % Leucocytes 10 500/μl with 16 % immature cells. The peripheral blood contained 21 normoblasts per 100 nucleated cells. Erythrocyte picture showed aniso- and poikilocytosis. Thrombocytes 38,000/μl. Haptoglobin 58 mg% Coombs test negative. No demonstrable thrombocyte agglutinins. Serum bilirubin 1 1 mg% Serum uric acid 12.2 mg % B.M.R. 153 E.S R. 53 mm/h. Biopsy from the iliac crest showed collagenous connective tissue and remains of compact and spongy bone tissue. Very small medullary cavities were seen in the latter containing cell poor bone marrow Tagging erythrocytes with Cr[51] showed a considerably reduced life-time the half life being 8 1/2 days. Surface measurements over the spleen liver and precordium showed a rise in the spleen/precordial ratio from 1 19 to 2 41 in the course of 15 days and a liver/precordial ratio from 0.42 to 0 65. The surface measurements thus suggested splenogenic haemolysis. During hospital stay the patient developed an attack of left-sided renal colic with passage of a stone.

Splenectomy was then decided upon in view of the considerable haemolysis and the severe thrombocytopenia. The patient received transfusions, and operation was then performed on the 29th October 1962 in Surgical Department D The spleen weighed 1 400 g and measured 22 × 14 × 9 cm Microscopical examination showed considerable extramedullary haemopoiesis, changes corresponding well to the findings in myelofibrosis-sclerosis. Post-operative course was uneventful.

Table I

Pat. no	Indications for splenectomy	Result of splenectomy		Died after splenectomy
		Hb concentration	Thrombocytes	
1	Thrombocytopenia	Normal before op.	++	—
2	Haemolytic anaemia, thrombocytopenia	+ to ++	++	—
3	Haemolytic anaemia, thrombocytopenia	+	(+)	+
4	Haemolytic anaemia, thrombocytopenia	+	—	—
5	Haemolytic anaemia, thrombocytopenia	++	++	—
6	Haemolytic anaemia, thrombocytopenia	++	++	—

— no, (+) slight, + definite, ++ marked improvement.

The patient felt considerably better after the operation. However, there was quite pronounced and persistent gastro-intestinal haemorrhage. In spite of intensive studies (repeated roentgenography of the gastro-intestinal canal, endoscopy and string-test) the aetiology of the haemorrhage could not be found. Despite the haemorrhage, the Hb concentration remained constant at 9–10 g% Leucocytes rose to 20,000–25,000/μl, still with considerable shift to the left (up to 11 % immature cells). There were about 75 normoblasts per 100 nucleated cells in the peripheral blood. Platelets rose to 196,000/μl (immediately prior to operation they were 11,000/μl). Reticulocytes 30–100 ‰. E.S.R. 21 mm/h. B.M.R. fell to 116 %. There was now no haemorrhagic diathesis. Cr^{51} measurement was repeated and the half-life value of 17 days obtained, still reduced but now considerably less than before splenectomy when it was 8 1/2 days. It was not until about 2 1/2 months after splenectomy that the Hb began falling because of loss of blood from the intestinal canal. As the aetiology of this could not be found, explorative laparotomy was performed three months after splenectomy. A duodenal ulcer was found and laparotomy with Billroth I gastrectomy and transabdominal vagotomy was performed. There was diffuse oozing of blood post-operatively the patient collapsed and death ensued.

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Since discharge, the patient has been admitted at times to a psychiatric department (Department E, Sct. Hans Hospital) for abuse of alcohol. After the splenectomy, the patient has been completely well somatically, and the haematological values have become normal. As of March 1963 – 21 months after the splenectomy – the patient still feels well. Hb 14.9 g%. Leucocytes 6 500/µl with only slight lymphocytosis, moderate shift to the left, and a few normoblasts in the peripheral blood. Erythrocyte picture shows anisocytosis. Platelet count approx. 200 000/µl. No haemorrhagic diathesis.

Comments

Splenectomy was performed on the following indications: 1. Severe anaemia with a considerable haemolytic component: continuous need for transfusion, reduced erythrocyte life time, very low haptoglobin, and slight reticulocytosis. 2. Pronounced thrombocytopenia. 3. Severe leucopenia, particularly neutropenia (leucocyte counts as low as 500/µl). 4. Rather pronounced discomfort from the enlarged spleen. Nearly two years after the splenectomy the patient feels completely well. Hb-concentration is normal. Platelet count is normal, there is no haemorrhagic diathesis. Leucocyte count is also normal, with only slight shift to the left.

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cerebral vessels and the pulmonary and portal veins, with lethal outcome. Simultaneously with the thrombocytosis there is often also increased haemorrhagic diathesis (9). Meilleur and Meyers (22) have shown, however, that small doses of radioactive phosphorus can control the thrombocytaemia just as busulphan could also be used to advantage in such cases.

In several cases, a considerable degree of hepatomegaly developed following splenectomy (3, 9, 13, 18, 24, 25). The hepatomegaly can become enormous, giving portal hypertension with haematemesis because of oesophageal varicosities (17). In such cases, myeloid metaplasia of the liver tissue will be found at autopsy.

Considerable leucocytosis is very often found in the peripheral blood following splenectomy. There is no agreement on the aetiology of this condition. It must be assumed to involve the loss of the bone marrow-inhibiting factor in the spleen. There are no side-effects from the leucocytosis.

An alternative to splenectomy is therapy in the form of adrenocorticosteroids, androgenic hormones and splenic irradiation, possibly over a restricted field of the organ. Systemic cytotoxic treatment, for example with busulphan, might be considered in certain situations. At the present stage it is not possible to lay down hard and fast lines for when medical therapy should be attempted, and when splenectomy. Tentative medical therapy is often necessary in the individual patient, possibly leaving the question of splenectomy to be decided by the results derived from cautious irradiation of the spleen. In these patients the therapy administered must be to a high degree individual and their centralised treatment is therefore desirable to permit the collection of adequate experience at those centres where there is an interest in the disease, and where the technical equipment is available which is necessary for an evaluation of the genesis of the anaemia and thrombocytopenia to as precise a degree as possible.

Summary

An account is given of six patients with myelofibrosis who underwent splenectomy. Only one of the patients died postoperatively, death being due to cardiac and liver insufficiency. Five of the patients had haemolytic anaemia, and there was a rise in Hb in all five. All six patients had thrombocytopenia, and a considerable rise in the thrombocyte count was obtained in four. None of the patients developed an exacerbation of their haematological condition.

The conclusion is that in most cases of myelofibrosis with severe haemolytic anaemia and/or thrombocytopenia, an improvement is observed in the form of higher trombocyte count, disappearance of the haemorrhagic diathesis, rise in Hb or alternatively a lesser need fo transfusions. The sole risk appears to be that associated with the operative intervention itself.

The indications for splenectomy in myelofibrosis and the possible complications, are discussed briefly.

Acknowledgement

I wish to thank the heads of the departments from which I have had kind permission to use case records and autopsy reports.

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athesis disappeared The basal metabolism fell and the serum uric acid became normal. The result was thus satisfactory haematologically A bleeding peptic ulcer however necessitated laparotomy and gastrectomy Following operation the patient died from haemorrhagic diathesis not completely explained by thrombocytopenia, as terminally the platelet count lay around 50 000—70 000/μl

Table I shows the indications for splenectomy in the six patients, together with the effect of the operation upon the Hb-concentration and the thrombocyte level

Discussion

Only one of these six subjects died postoperatively following splenectomy This was patient No 3 who had a considerably impaired liver function and who died in cardiac and liver insufficiency after developing a subphrenic abscess Five of the six subjects exhibited haemolytic anaemia The Hb was found to rise in all five, the rise being most pronounced in patients Nos 2 5 and 6 All six patients had thrombocytopenia. A considerable rise in thrombocyte count was found in four patients while the thrombocyte count remained unchanged or insignificantly altered in two. Not one of the patients presented a deterioration of the haematological condition Definite hepatomegaly was observed only in patient No 2 It is uncertain whether the hepatomegaly was accentuated as a result of the splenectomy as the entire progress of the disease in this patient took a rapid and violent course. Two of the patients died from other causes shortly after operation Patient No 4 died from bronchopneumonia two months after operation Patient No 6 died following gastrectomy three months after the splenectomy The gastrectomy was performed for a bleeding ulcer

The tendencies suggested in this present limited material are therefore as follows In patients with myelofibrosis and severe haemolytic anaemia and/or thrombocytopenia, the condition is not exacerbated by splenectomy On the contrary in most cases the condition improves, in the form of a higher platelet count, disappearance of the haemorrhagic diathesis, rise in Hb or alternatively reduced transfusion requirements. The sole risk thus appears to be that associated with the actual operation which is in line with the experience of others (6 13)

In a number of communications (2 6, 24 25 32) the following are suggested as indications for splenectomy 1 Severe haemolytic anaemia. 2. Severe thrombocytopenia. 3 Pronounced discomfort from the enlarged spleen or recurrent splenic infarctions 4 Severe hypermetabolism, with sweating and considerable loss of weight It is often difficult to determine the origin of the anaemia. There may be either reduced production or increased destruction of erythrocytes, or often a combination of these two factors. In many cases a correct evaluation of the degree of the haemolysis will need a determination of the life-time of the erythrocytes, e g by Cr^{51} tagging (24 30) By combining this with measurements of the radioactivity over the spleen and liver it is possible in a number of cases to estimate the role of the spleen for the destruction of erythrocytes

Numerous complications of splenectomy have been described in myelofibrosis. In some cases the splenectomy is followed by thrombocytosis with megakaryocytes in the peripheral blood (13 17) resulting in thrombosis (9 25) especially of the

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From the Department of Microbial Diseases (Head W R. O Gorlings, M. D) and the Department of Chest Diseases (Head J Swierenga, M. D) University Hospital, Leyden, The Netherlands

The Relationship of Results of Sputum Cultures to Clinical Effect in a Controlled Trial of Continuous Antibiotic Treatment of Chronic Bronchitis with Demethylchlortetracycline or Penicillin V

By

R J Djajadiningrat J Hrouda and W R. O Goslings

Previous work has suggested that antibiotics, given over a long period, give an improvement in the clinical symptoms of chronic bronchitis (1 2, 3 12, 16, 17)

As the action of antibiotics in the human body is only or mainly antibacterial, the reason for such beneficial action can only be found in any antibacterial action produced by these substances on those microorganisms, which are the main cause of infection in chronic bronchitis, e. g H.-influenzae and D.-pneumonia (4 11 13, 14 15, 20)

Nevertheless there is still uncertainty whether the suppression of pneumococci or of H.-influenzae is the main reason for any beneficial results obtained by long term antibiotic therapy in lower doses. Murdoch et al. (16) and Norman et al (17) saw reduction of both H. influenzae and pneumococci coincident with reduction of exacerbations of the disease with a dose of 1 gram of one of the tetracyclines daily

The report of Frances et al. (5) on the results obtained with one of the tetracyclines and with penicillin-V suggests that the clinical results could be explained by accepting a suppressive effect on pneumococci only

The aim of the present trial was to relate the effect of antibiotic prophylactic treatment on the sputum-flora to the effect on the clinical symptoms. The dosage of the penicillin V (1 000 mg alternate days) was chosen because it was expected that with this dose pneumococci only would be suppressed, as H.-influenzae is somewhat resistant to the action of this form of penicillin (6, 7 10) the demethylchlortetracycline in the dose of 600 mg alternate days on the other hand might in addition suppress H.-influenzae.

Methods

The experiment reported here was double-blind controlled trial, which was held from October 16, 1960 until April 16, 1961 The

Submitted for publication September 30, 1963.

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As the action of antibiotics in the human body is only or mainly antibacterial, the reason for such beneficial action can only be found in any antibacterial action produced by these substances on those microorganisms, which are the main cause of infection in chronic bronchitis, e. g H.-influenzae and D pneumonia (4 11 13 14, 15 20)

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The aim of the present trial was to relate the effect of antibiotic prophylactic treatment on the sputum-flora to the effect on the clinical symptoms. The dosage of the penicillin-V (1 000 mg alternate days) was chosen because it was expected that with this dose pneumococci only would be suppressed, as H.-influenzae is somewhat resistant to the action of this form of penicillin (6 7 10) the demethylchlortetracycline in the dose of 600 mg alternate days on the other hand might in addition suppress H.-influenzae.

Methods

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Table I Mean of highest DMCT and penicillin-V blood levels (γ/ml) found during first 3 hours after administration

	mg	Mean	95% limits
DMCT	150	3.45	2.80—4.10
DMCT	300	3.92	3.27—4.57
Penicillin V	250	1.57	1.21—1.93
Penicillin-V	500	4.17	3.05—5.29

No antibiotic activity was found in any of the serum samples of 32 patients using the placebo s.

trial was conducted on outpatients who had suffered from persistent cough with sputum for the past year (the majority of them were known to us for already more than two years). For the purposes of this trial, the patient should have had at least one period in which an exacerbation had caused loss of his ability to work for some time. No patient entered the trial when the disease had progressed so far that he was permanently unable to work, a history of penicillin sensitivity was present, the chest X ray had not excluded other lung diseases and if he had suffered from typical exacerbations of bronchial asthma for the past year.

For the purposes of this investigation the severity of each exacerbation was graded as follows:

Grade 1 — one or more of the following symptoms: increased volume of sputum, increased purulence of sputum, fever, increased frequency of productive coughing.

Grade 2 — the same as grade 1 with interruption of daily routine or work out of doors.

Grade 3 — the same as grade 1 with the patient bedridden at home.

Grade 4 — the same as grade 1 but severity of symptoms necessitating admission in a hospital.

In total 96 patients were included in the trial and allotted at random to 6 groups by the use of random permutations. They received in identical capsules:

A — demethylchlortetracycline (DMCT) 150 mg given four times every second day

B — demethylchlortetracycline (DMCT) 300 mg given twice every second day

C — penicillin-V 250 mg given four times every second day

D — penicillin-V 500 mg given twice every second day

E — saccharum lactis, 250 mg given four times every second day

F — saccharum lactis, 500 mg given twice every second day

At the pre trial interview each patient was carefully instructed. He was given a graduated sputum flask and was asked to make a record of the volume and the colour of the 24-hour sputum once weekly (and if possible daily during exacerbation of the bronchitis). The number and duration of attacks were also recorded.

The cooperation of the general practitioners was sought. When a patient showed a severe exacerbation the general practitioner was free to prescibe whatever treatment he thought necessary at the moment. He was asked, however, to inform us of the chemotherapy prescribed and to restart the patient on the capsules supplied by our clinic after cessation of their treatment. Both the general practitioners and the patients were asked to send sputum to our laboratory for culture before and after the completion of the treatment of an exacerbation by the general practitioner. It must be said, however, that this was not always done.

Once a month every patient visited our department for a physical check up. The patient was instructed to collect all his sputum of that morning from awakening until the time he came to the office. The volume of this sputum quantity was measured, its colour noted and a full bacteriological examination was made.

In a number of patients blood serum levels were examined one and three hours after the administration of 150 mg and 300 mg DMCT and of 250 mg and 500 mg penicillin-V. The concentrations of DMCT were assayed by the agar diffusion method using Bacillus cereus var mycoides ATCC 9634 as the test micro-organism, the serum penicillin-V levels by a dilution method employing Streptococcus haemolyticus group A Lancefield as the test micro-organism.

The bacteriological examination of the sputum was done according to the technique of Mulder (15). The rate of growth was denoted in a standardized way by the number 0 up to and including 4, in which 0 stands for no growth and 4 for maximal growth. Sputum was also examined for its

able II Average sensitivity of H-influenzae- and pneumococcus strains to penicillin-V and DMCT

	Penicillin-V (strains isolated from sputa from patients in penicillin-V groups)		DMCT (strains isolated from sputa from patients in DMCT groups)	
	No. of strains examined	Pen. V inhibition conc. (γ/ml)	No. of strains examined	DMCT inhibition conc. (γ/ml)
H-influenzae	25	Average 8.72 (95% limits 6.70—10.74)	51	Average 0.98 (95% limits 0.84—1 12)
Pneumococci	17	0.25 (lowest conc. used)	17	Average 0.79 (95% limits 0.56—1.02)

contents of eosinophilic cells according to the method described by Mulder (13)

Sera obtained at every monthly investigation and before and after the exacerbation were preserved in frozen state for serological examination for possible viral infection.

Serum protein determinations were made by paperelectrophoresis at the beginning of the trial.

Results

Of the 96 patients enrolled in the trial, 8 were excluded from statistical analysis for the following reasons

1 One in the placebo-group because of serious deterioration of clinical status.

2. One in the penicillin-group because it became known afterwards that she had previously had attacks of asthma and was taking prednisone prescribed by the family physician continuously She therefore did not fulfil the criteria for inclusion in the trial.

3 One in the DMCT-group because of a possible drug sensitivity to DMCT This patient had never any signs of asthmatic attacks previously but developed typical asthmatic attacks on the da he took the capsules, from the third week on Attacks subsided after discontinuation of the drug.

4 Two patients in the DMCT-group and three in the placebo-group were withdrawn because they were not co-operative.

Despite random selection more young people had found their way into group D However there was no significant difference in the average age of the six groups. There was no bias in the incidence of H.-influenzae in the sputum at the pre-treatment examination. Regarding the incidence of pneumococci, group A showed by far the lowest incidence. The severity of the clinical state and the history of exacerbations in the previous winter were well scattered.

Four patients had a hypogammaglobulinaemia (lower than 0 6 g %) one patient in group A, two patients in group C and one patient in group D

Serum concentrations and sensitivity of cultured H -influenzae- and pneumococcus strains

The mean of the highest serum concentrations of the antibiotics used found during the first three hours following the administration of 150 mg DMCT (group A) 300 mg DMCT (group B) 250 mg penicillin-V (group C) and 500 mg penicillin-V (group D) is shown in table I

The sensitivities of isolated H influenzae and pneumococcus strains to DMCT

Table III Pneumococcus-flora in sputum during treatment

Average grade of growth	No. of patients								
	A	B	C	D	E	F	A+C	B+D	E+F
0	8	13	8	13	4	7	16	26	11
0–1	4	2	6	3	3	4	10	5	7
1–2	1	1	1	0	5	1	2	1	6
2–3	0	0	0	0	1	3	0	0	4
Total no. of pat.	13	16	15	16	13	15	28	32	28

Table IV Incidence of pneumococcus before and during treatment

Groups	No. of patients with sputa containing pneumococci/no. of patients examined						
	Treatment before	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 6 months
A	2/13	0/13	1/13	2/13	0/12	2/11	2/12
B	7/15	2/16	1/16	0/16	0/16	1/15	2/16
C	6/13	4/15	1/13	1/12	1/12	2/14	2/15
D	5/14	0/16	0/14	1/16	0/16	0/16	2/16
E	6/11	8/13	6/13	6/12	6/13	4/12	5/13
F	7/11	6/15	5/15	4/13	5/13	1/13	5/15

(group A and B) and penicillin V (group C and D) are summarized in table II.

It can be deduced from these data that the peak blood levels of DMCT in the patients using this drug in this study were high enough to suppress both H. influenzae and pneumococci. In this regard it seems worth mentioning that Knothe (9) and Sweeney et al. (21) showed that the serum levels of DMCT six hours after a dose of 150 mg or 300 mg may be even higher. These were not tested in this study.

The blood levels in the patients using penicillin V in this study, as expected, were only sufficient to suppress pneumococci, but inadequate for suppression of H. influenzae. One has to take into consideration of course that the height of the blood level of the drug is not necessarily the determinant for the effect on infections in tissues, as the tissue levels of antibiotics are in general lower than blood levels. One has also to consider the fact that in this study the antibiotics were only given every other day, so that temporarily suppression is not followed necessarily by elimination.

Sputum bacteriological data, character and volume

Pneumococci

There was sometimes a definite variation in the rate of growth from the various monthly samples of the same patients, while in a few patients some monthly samples were not examined. We calculated therefore the average rate of growth of all monthly samples provided during the period of the study for each patient.

In table III the number of patients in each subgroup with the stated average rate of growth of pneumococci from the sputa collected during the experiment are given.

As can be seen from table III the average rate of growth was less in groups A, B, C and D (treated groups) than in groups E and F (placebo-groups). The strongest suppression of pneumococci is seen in groups B and D which resemble each other very markedly, a lesser one for groups A and C, which again resemble each other very closely. Because of the small numbers in the groups, making a useful further statistical evaluation difficult, groups B and D on the one hand and A and C on the other hand, were combined on the basis of their close resemblance and compared with the placebo-groups E and F. This seemed the more acceptable as the whole purpose of the trial was to find out whether definite correlation could be found between the grade of suppression of micro-organisms and clinical results. To make such a comparison it was thought advisable to compare groups which showed statistically significant differences regarding this suppression of microorganisms. Results of the combined groups are also given in table III.

The differences between the treated groups and the placebo-groups are statistically significant $(0.01 > P > 0.005)$. From the figures it can be seen that the treated groups (A + C and B + D) were much better than the control group (E + F). A comparison between group B + D and group A + C shows that the trend in favour of group B + D is important. This difference is nearly statistically significant at the 5 [%] level.

I[n] table IV the data on the number of patients with sputum containing pneumococci against the total number of

Table V H-influenzae-f[ound] in sputum during treatment

Average grade of growth	No. of patients					
	A	B	C	D	E	F
0	8	8	4	6	4	6
0–1	1	3	4	3	3	1
1–2	1	2	5	6	5	5
2–3	3	3	2	1	1	3
Total	13	16	15	16	13	15

patients examined before treatment and at each monthly investigation during treatment are given.

From this table it can be seen that the frequency of isolation of pneumococci in patients of group B (treated with 300 mg DMCT b. i. d. every second day), group C (250 mg penicillin-V q. i. d. every second day) and group D (500 mg penicillin-V b. i. d. every second day) decreased already during the first two months and remained at this low level for the following four months.

The incidence during the pre-treatment period of pneumococci in the patients allotted to group A was already so low that their appearance or disappearance during treatment could not be judged to be due with certainty to the drug effect. The placebo-groups E and F did not show a marked decline in the frequency of positive isolation. Furthermore it can be stated also that the number of pneumococci cultured from the monthly sputa of the patients with positive cultures did not change markedly.

H.-influenzae

The data on H.-influenzae were compiled in the same way as for pneumococci (table V).

Table III Pneumococcus-flora in sputum during treatment

Average grade of growth	No. of patients								
	A	B	C	D	E	F	A+C	B+D	E+F
0	8	13	8	13	4	7	16	26	11
0–1	4	2	6	3	3	4	10	5	7
1–2	1	1	1	0	5	1	2	1	6
2–3	0	0	0	0	1	3	0	0	4
Total no. of pat.	13	16	15	16	13	15	28	32	28

Table IV Incidence of pneumococcus before and during treatment

Groups	No. of patients with sputa containing pneumococci/no. of patients examined						
	Treatment before	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 6 months
A	2/13	0/13	1/13	2/13	0/12	2/11	2/12
B	7/15	2/16	1/16	0/16	0/16	1/15	2/16
C	6/13	4/15	1/13	1/12	1/12	2/14	2/15
D	5/14	0/16	0/14	1/16	0/16	0/16	2/16
E	6/11	8/13	6/13	6/12	6/13	4/12	5/13
F	7/11	6/15	5/15	4/13	5/13	1/13	5/15

(group A and B) and penicillin V (group C and D) are summarized in table II

It can be deduced from these data that the peak blood levels of DMCT in the patients using this drug in this study were high enough to suppress both H influenzae and pneumococci. In this regard it seems worth mentioning that Knothe (9) and Sweeney et al. (21) showed that the serum levels of DMCT six hours after a dose of 150 mg or 300 mg may be even higher These were not tested in this study

The blood levels in the patients using penicillin V in this study as expected were only sufficient to suppress pneumococci, but inadequate for suppression of H. influenzae. One has to take into consideration of course that the height of the blood level of the drug is not necessarily the determinant for the effect on infections in tissues, as the tissue levels of antibiotics are in general lower than blood levels. One has also to consider the fact that in this study the antibiotics were only given every other day so that temporarily suppression is not followed necessarily by elimination.

Sputum bacteriological data, character and volume

Pneumococci

There was sometimes a definite variation in the rate of growth from the various monthly samples of the same patients, while in a few patients some monthly samples were not examined We calculated therefore the average rate of growth of all monthly samples provided during the period of the study for each patient

that group A distinguished itself from the other groups in so far that it contained at the pre treatment examination by far the lowest number of patients carrying pneumococci, which could have exerted a favourable effect on the incidence of exacerbations. This possibility seems to be supported by the fact that the mean severity of the few exacerbations in group A could be more easily compared with the mean severity of exacerbations in group C and also with the mean severity of exacerbations in groups E and F than those in groups B and D

Group A showed a mean severity of exacerbation of 1.94 the severity of an exacerbation being denoted by the numbers 1—4 according to the four grades. Groups B, D and E showed severities of 1.40, 1.58 and 1.78 respectively while groups C and F the values of 2.24 and 2.44

The number of patients in each group was too small for further useful statistical evaluation of the data given and it seemed advisable to combine various treatment groups and placebo-groups. As mentioned in the introduction of the report, a reasonable explanation of any clinical benefit noted seems only possible on the basis of suppression of those bacteria, which have a causal relationship to the clinical picture of chronic bronchitis.

In this study a statistically significant difference was found only in the suppression of pneumococci, not in any action on H.-influenzae. It seemed therefore justifiable to analyse the clinical results according to the grouping already used for the evaluation of the measure of suppression of pneumococci.

Table VII shows the incidence of exacerbations for the groups. The mean severity of exacerbation for group B + D was 1.50 that for group A + C, 2.13

Table VII Incidence of exacerbations for groups combined according to suppression of pneumococci

	No. of patients		
	B+D	A+C	E+F
Total no.	32	28	28
Without exacerb.	17	9	6
With exacerb.	15	19	22
With 4 exacerb.	1	1	2
With 3 exacerb.	2	3	0
With 2 exacerb.	2	7	8
With 1 exacerb.	10	8	12

while group E + F gave a similar figure of 2.14

As can be seen from this table the number of patients without exacerbations and the distribution of the patients with exacerbations according to the number of exacerbations in each patient are much more favourable for the group B + D than for the groups A + C and E + F the latter ones not differing very much from each other The differences between groups B + D A + C and E + F were, however only significant at the 10 % level The difference between the group B + D and the combined groups A + C + E + F is significant at about the 5 % level.

Although the differences in the incidence of exacerbations are not clearly significant, the differences in the mean severity of the exacerbations between the combined groups B + D A + C and E+F are statistically significant ($0.005 > P > 0.001$)

On the other hand one can of course combine the results according to the type of antibiotic given. It must be realized, however that in this case there are no differences at all between the combined groups A + B and groups C + D re-

Table VI Incidence of exacerbation in various experimental groups

	No. of patients					
	A	B	C	D	E	F
Total no.	13	16	15	16	13	15
Without exacerbation	6	9	3	8	3	3
With exacerbation	7 (54%)	7 (44%)	12 (80%)	8 (50%)	10 (77%)	12 (80%)
With 4 exacerbations	0	1	1	0	1	1
With 3 exacerbations	1	1	2	1	0	0
With 2 exacerbations	2	2	5	0	2	6
With 1 exacerbation	4	3	4	7	7	5

In contrast to the results obtained regarding pneumococci no significant differences could be seen in the average rate of growth of H. influenzae in the various experimental groups. The distribution of the patients with different average grades of growth in the six groups was without any special trend Combination of treatment groups A and B C and D and the two placebo-groups still did not give any better results, nor any other combination It can be stated also that the percentage of positive cultures at each examination period showed no definite decrease or increase in comparison with the pre treatment figures over the six month treatment period. Our conclusion therefore *is that no significant reduction in the incidence of H* influenzae was obtained in any of the six experimental groups.

Sputum volume and character

The treatment régimes brought no reduction in volume and the degree of purulence (colour the grade in which leucocytes were present in the sputum smears stained with gram-stain or methyleneblue or the percentage of eosinophils in the wet preparations) It must be said, however that the degree of purulence was in general already only moderate at the pretreatment examination (mostly grade 1—2) As most of the sputum examinations were done at the monthly check ups and some of the more purulent sputa of the exacerbations were not examined any influence on the purulence during exacerbations was diluted by the more frequent examinations during the intermissions. However these data point to the fact that during the trial period there was no notable change in the base line condition of the patients, nor in the treatment nor in the placebo-group

EXACERBATIONS

Table VI *gives the number of patients* without exacerbations and the number with exacerbations divided according to the number of patients with respectively 4 3 and 2 exacerbations and 1 exacerbation throughout the study

As can be seen from this table groups B and D are again comparable regarding the percentage of patients with exacerbations while the placebo-groups E and F both show high percentages for patients with exacerbations. Group C equals groups E and F while group A resembles groups B and D It must be said however

groups had antibiotic treatment during exacerbations (mostly 5—7 days of one of the tetracyclines or of chloramphenicol in doses of q d. 0.5 g) In the placebo-groups E + F this happened 14 times, against 7 times in the DMCT-groups and 5 times in the penicillin-V-groups. As a therapeutic treatment with the dosages used during the exacerbations has also some prolongated effect on the presence of the micro-flora in the bronchial tree, preventing a direct recurrence of a new exacerbation, this might have influenced the number and the severity of exacerbations in the so-called placebo-groups to larger extent than in the treatment-groups. The difference between the continuous antibiotic suppressive treatment in the manner given and a "full placebo-group might therefore have been even larger than found now

If one accepts the standpoint that any benefit of antibiotic treatment can only be explained reasonably by its suppressive action on a micro-organism, the data found point to the fact that the clinical severity of the exacerbations in chronic bronchitis is mainly decided by the action of pneumococci and not by the action of H.-influenzae, as the results of the bacteriological examinations showed a significant reduction in the pneumococcal flora only and no significant changes in the presence of H.-influenzae. In this regard t seems worth mentioning that those treatment-groups (300 mg DMCT b d) and group D (500 mg penicillin-V b d) also showed the lowest mean severity of exacerbations still present. Furthermore both groups showed the best results regarding reduction in the incidence of the exacerbations, a reduction, which became nearly significant when these two fully comparable groups were combined That such a significant reduction in severity and incidence was not seen when the two next best groups regarding the suppression of pneumococci (group A, 150 mg DMCT q i. d. and group C, penicillin V 250 mg q i. d.) were combined could point to the fact that the suppression of pneumococci in patients with chronic bronchitis should be nearly absolute before a definite clinical benefit is seen. This degree of suppression seems to be reached more easily by giving the total daily dose of the antibiotics used in two doses only (morning and evening) rather than in four doses spread out over the whole day

If one looks at the results only from the standpoint of the antibiotics used without regard to the bacteriological effects obtained, the results of the combined groups using DMCT regarding the mean severity of the exacerbations were better than for the combined groups using penicillin-V No such preference could be noted, however regarding the incidence of the exacerbations. It seemed possible that the increased effect of the combined groups of DMCT regarding the mean severity over the combined groups of penicillin-V might have been influenced by a more favourable composition of treatment group A (DMCT 150 mg q i. d.) By chance this group A contained at the pre-treatment examination by far the lowest number of patients having pneumococci in their sputum before the therapeutic trial started.

As mentioned, no significant difference could be noted in any of the treatment groups regarding th volume or the degree of purulence of the sputum. This is understandable from the bacteriological results mentioned. It has been shown by Mulder et al (14) that as long as H influenzae is present, the sputum in patients with chronic bronchitis remains

Table VIII Incidence of exacerbations for groups combined according to type of antibiotic used

	No. of patients		
	A+B	C+D	E+F
Total no.	29	31	28
Without exacerb.	15	11	6
With exacerb.	14	20	22
With 4 exacerb.	1	1	2
With 3 exacerb.	2	3	0
With 2 exacerb.	4	5	8
With 1 exacerb.	7	11	12

garding the bacteriological results obtained. Any difference to be found between these groups is then of course not based on differences in suppression of micro-organisms. The combination of the various experimental groups in this way gives the data represented in table VIII

Although the combined group A + B (DMCT-group) does show a somewhat more favourable trend in the number of patients showing no exacerbation at all and in the distribution of the patients with exacerbations according to the number of exacerbations seen in each patient than the combined groups C + D and E + F there is no statistically significant difference at all between the three groups (0 5>P>0 3) One has to take into consideration furthermore that the more favourable trend for the combination A + B than for the combination C + D is partly due to the data for group A, which might have been a favourable group initially

However the differences in the mean severity of the exacerbation between groups A + B, C + D and E + F (respective values 1 68 1 98 and 2 14) is statistically significant (0 05>P>0 02) in this order of sequence.

Side effects

Apart from one case of a possible drug induced attack of asthma in a patient using DMCT no side effects were noted.

Virological data

Sera were examined in complement-fixation tests against antigen of para-influenza I (Senday) parainfluenza II (Firth) para-influenza III Coe-virus and Adeno-virus. A fourfold or greater rise in titre was found 18 times (1 against para-influenza I 6 against para influenza II 1 against para-influenza III 1 against Coe-virus 9 against the group of A.D viruses) but only 7 of the 18 rises could be correlated with the clinical symptoms of an exacerbation (2 against para-influenza II 1 against para-influenza III and 4 against the group of A.D -viruses) Eighty eight of the exacerbations showed no correlation with any of the types of viral infections examined in this way In none of the patients could a significant rise in antibody titre to the influenza A or B viruses (haemagglutination inhibition test) be demonstrated.

Discussion

The results show that even using small doses of one of the antibiotics demethylchlortetracycline or penicillin-V (total dose 1 g a day) only every other day during the 6 months of the "winter" period one can obtain a statistically significant reduction in the severity of the clinical exacerbations during that winter period in patients suffering from chronic bronchitis, compared with groups of such patients receiving no continuous antibiotic treatment

In this regard it should be mentioned that patients of the placebo-group" were not entirely free from antibiotic treatment during the trial period as the family doctor was allowed to give any treatment he thought necessary when his patient showed an exacerbation Consequently several patients in the placebo-

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mucopurulent and in this trial no significant reduction in H. influenzae was seen in any of the treatment-groups. This brings up the point whether continuous antibiotic treatment of chronic bronchitis mainly suppressing pneumococci only and for that reason giving the patient a state of clinical well being has also any beneficial effect in the long run for the patient. As we all know these patients do not die so much from the infection per se as from the after effects of the obstructive emphysema which develops in nearly every one of them. As one of us has pointed out, however (7) it is still too early to decide whether the development of this obstructive emphysema is mainly due to the deleterious effect of the continuous presence of H. influenzae in the mucosa of the bronchial tree (8) or to the effect of pneumococci causing perhaps the *minimal bronchopneumonial infiltrations* around the terminal bronchioli which Reid (18, 19) sees as the cause of the obliteration of the terminal bronchioli that she has noted in her histological preparations. As long as this has not been decided continuous antibiotic suppressive treatment in patients with chronic bronchitis, even if it suppresses pneumococci only still seems worthwhile.

Summary

Eighty-eight patients with moderately severe chronic bronchitis took part in a double-blind controlled therapeutic trial using demethylchlortetracycline (total daily dose 600 mg alternate days) penicillin V (total daily dose 1 g alternate days) and a placebo (alternate days). The benefit of antibiotic treatment was demonstrated by a significant suppression of the pneumococcal flora in the patient's sputum. No significant differences could be found regarding the presence of H. influenzae during the trial period. The treated groups showed a significant improvement in the severity of the exacerbations. The results obtained with DMCT did not differ significantly from those obtained with penicillin V. It seems preferable to give the total daily dose of DMCT and penicillin V in two divided doses and not in four divided doses.

The findings suggest that the good results obtained with prolonged antibiotic suppressive treatment in chronic bronchitis are, at least partly due to a suppressive action on pneumococci. It is however impossible to exclude from the data of this study that added suppression of H. influenzae would not improve total results.

Acknowledgements

We are indebted to Dr. J. Versteeg M. D., Laboratory of Microbiology University of Leyden, and Dr. N. Masurel, M.D. Laboratory for Influenza Research, The Netherlands Institute of Preventive Medicine Leyden for performing the virological analyses and to Mr. H. de Jonge, The Netherlands Institute of Preventive Medicine, Leyden, for the statistical analyses of the results.

We are grateful to Lederle Laboratories Division American Cyanamid Co., Pearl River New York, for the DMCT and to Mycofarm Delft, Division of the Royal Netherlands Fermentation Industries, Ltd., Delft, for the penicillin-V the placebo-material and for dispensing all three substances. We also thank these two firms for a grant to cover expenses of the statistical analysis.

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Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From the Hospital for Infectious Diseases, Uppsala, Sweden

An Extensive Outbreak of Gastroenteritis Caused by Salmonella Newport

I. Some Observations on 745 Known Cases

By

Bo Bille, Tore Mellbin and Folke Nordbring

All over the world *Salmonella Newport* is a common cause of gastroenteritis (28, 29 32, 34) Several reports from recent years on outbreaks of infections with this organism are to be found in the literature (5 10 11 14 20, 25 30) No major epidemic has been known in Sweden since the report by Eriksson in 1941 (9)

Although S Newport gives rise predominantly to the gastroenteritis syndrome, there is evidence from the literature that this type of salmonella not infrequently penetrates into the blood stream, causing septicaemia and a syndrome similar to enteric fever Borustein (4) noted this tendency in 1943. Many instances of peracute course with a fatal outcome have been described (5 8, 11 14 20 30) and Saphra (27) has found a mortality rate of 4 1 per cent in a selected material Focal manifestations have been observed, such as meningitis (7 24) and osteomyelitis (16)

The capacity to produce a generalized disease and not only a more or less mild gastroenteritis is common t many salmonellae. Differences in this respect exist between various salmonella types (6 13 15 21) It has been stressed in the papers referred to previously that S New port has a marked pathogenicity This tendency is also apparent from the experiments on human beings by McCullough and Eisele (18, 19) in which comparatively small numbers of S New port organisms administered by mouth in some instances produced symptoms.

The pattern of clinical illness which ensues from consumption of food contaminated with salmonellae may depend upon such factors as the dose of bacteria ingested individual susceptibility and the type of salmonella. The duration of the period of excretion of organisms in the faeces may also vary with the type of salmonella. It has been mentioned that S. Newport tends to remain for many weeks in a good proportion of cases (10 14 20)

It seems to us to be important that the characteristics of an infection with a given type of salmonella should be

Submitted for publication October 8, 1963.

Table I. Types of care of 745 known cases of salmonellosis due to S. Newport, including bacillary carriers

Designation	Residents of Uppsala	Type of care	No.
Group A	Yes	At the Uppsala Hospital for Infectious Diseases	416
Group B	Yes	At home, supervised by mobile public-health team	175
Group C	Yes	At an adjacent infectious unit	35
Group D	Yes	At institutions or otherwise	47
Group E	No	At the Uppsala Hospital for Infectious Diseases	72

than 200 cases with symptoms of disease (fig. 1, lower part). Over and above these, a growing number of milder cases and bacillary carriers were recorded, since the epidemic led to comprehensive bacteriological investigations of employees in food-production industries, provision stores and restaurants, of military personnel, of children in schools, and of family contacts.

Under Swedish law all persons with positive faecal culture of salmonella must be isolated compulsorily in an infectious unit. The rapid accumulation of new cases gave rise to great demands for beds at our hospital and special arrangements had to be made. Thus, two departments for the care of the chronically ill, one rehabilitation unit and one school for mentally retarded children were temporarily evacuated and prepared to receive patients with salmonella infection. A number of patients were referred to infectious units in neighbouring towns. However these measures were insufficient, and care in the home was organized for especially mild cases and carriers living under satisfactory housing conditions. The patient at home received printed instructions concerning hygiene, and medical care was supervised by a team consisting of a doctor and a public-health officer.

Although the number of traced cases was largest in the second week of May (up to 50 new cases each day), the epidemic did not ... definitely until the second week of June (2). After that time some scattered cases were discovered, the last on Sept. 5th.

Material

Number of cases

Four hundred and eighty-eight patients were admitted to the Uppsala Hospital for Infectious Diseases with its temporary annexes. Thirty-five patients were admitted to adjacent infectious units, and 222 patients were taken care of in their homes, in the military barracks or otherwise. Altogether this amounts to 745 known cases, including bacillary carriers. Table I shows the distribution of the patients in the different types of care.

It is quite likely that the number of infected persons was much greater than that recorded above, many of them being undetected. Rundberg (26) estimated that 3,000 people were in contact with the contagious matter by eating the contaminated food. Some 50 persons from other parts of Sweden were known to be infected on a visit to Uppsala around May 1st. The total number of known cases is thus close to 800.

The 673 patients constituting groups A, B, C and D in table I in all probability comprise the total number of known cases among the residents of Uppsala. This number amounts to 9 per mille of the population of Uppsala at the time. It has been possible for us to study the groups A, B and C (626 cases) in some detail.

Distribution according to sex

Of the total Uppsala material (A, B, C, D) and of the material more thoroughly studied (A, B, C) 44.4 to 47.0 % of the infected persons were men and 53.0 to 55.6 % were women. The material is selected, inasmuch as the search for faecal carriers was concentrated on certain institutions, particularly provision shops. A high proportion of the employees of the latter are women. However the sexual distribution of the salmonella material did not deviate remarkably from the normal distribution of the population of Uppsala at the time (48 % male).

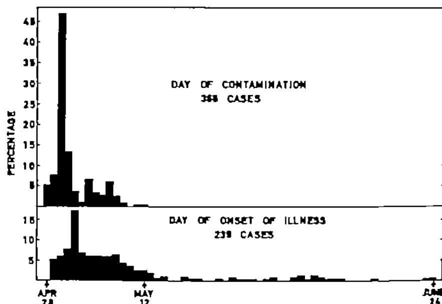


Fig 1 Upper part: the distribution on different days of consumption of infected pastry Lower part the distribution on different days of onset of illness.

clarified as completely as possible. We had the opportunity to make some observations on infection with S Newport during an extensive epidemic which occurred in the Uppsala region in 1960 There were 745 known patients in this area which makes the epidemic one of the largest due to S Newport hitherto reported. In this and the following papers some epidemiological clinical and serological data will be presented.

The epidemic

An outbreak of S. Newport food poisoning occurred at the end of April 1960 in Uppsala, a university town with a population at the time of approximately 76 000 It was soon established that the source of infection was butter cream, an ingredient of different types of pastry supplied by one single large bakery from the 28th of April to the 7th of May when the bakery was closed. A number of cakes were consumed in the next few days also, in one known instance as late as the 12th of May (fig 1 upper part) However the butter cream in question was first and foremost an integral part of a special cake with a pleasant flavour of lemon, intended for the festivities on April 30th, the day when all Sweden and particularly all Uppsala celebrates the coming of Spring Five hundred of these special cakes were distributed in a short time and each one was intended to be shared by several persons. In addition, some 450 contaminated cakes of other types were sold during the same short time (26)

The concentrated manufacture and supply of the cakes contributed to the explosive spread of the epidemic. The cakes were delivered not only to Uppsala itself but also to the surrounding countryside. As will be seen from fig 1 365 patients knew exactly the day they had consumed the infected pastry Approximately half of them had ingested it on April 30th.

Th butter cream was composed of butter margarine, sugar and eggs, and was not heated (26) A pure culture of S Newport was easily obtained in the laboratory from specimens of the butter cream. The primary source of infection was not definitely disclosed. It is true that eggs from the bakery were shown to be infected with S. Newport, but a large number of the employees of the bakery were subsequently found to be bacillary carriers, and some member of the staff might also have infected th cream.

During th first two weeks of the epidemic the health authorities were informed of more

distribution was observed between different age groups, as will be seen from table III. The age groups 1—6, 16—24 and 25—59 years follow the pattern mentioned above. The age group 7—15 years differs from the others, inasmuch as no less than 83.2 % were carriers. The explanation of the marked predominance of carriers among the school-children is probably the fact that the schools of the city were particularly carefully investigated in order to trace faecal carriers, and large number of carriers were in fact found. In contrast, proportionately few persons above 60 years of age were involved in the extensive bacteriological investigations carried out in various factories and shops. This may have had an influence on the proportions of carriers and patients with symptoms in the oldest age group, in which as many as 46.8 had severe or moderately severe symptoms. However the distribution may also illustrate the well-known and common clinical observation that elderly people tolerate a salmonella infection less well than younger adults. The only death in the present epidemic also occurred in the old age group.

Consumption of infected pastry

Of the 626 patients in the A, B, C material, 433 (69.2) stated that they had consumed cakes with butter cream from the bakery in question some time during the actual period (table IV). Moreover further 16.1 of the patients, who could not recall eating the cakes, had regularly bought provisions in stores to which the large bakery delivered its products. No less than 85.3 thus had direct contact with the stores supplied by the concern and with its products. Thirty-five cases (5.6) were considered to be probable secondary cases. No explanation whatsoever of the mode of contamination could be offered in 57 cases (9.1

I appears from table IV that the percentage of patients aware that they had consumed contaminated food is highest among the markedly ill. This relationship may simply be demonstration of the fact that ingestion of large dose of bacteria may result in severe illness. However the percentage of carriers who had eaten the cakes (65.8) may be too low because some of them did not remember in detail what actually happened some time ago, knowingly or not.

Table IV. The consumption of infected pastry by patients with different grades of illness (A, B, C material)

Grades of illness	Patients who had ingested infected pastry	
	No.	%
Severe	7	100.0
Moderate	118	76.1
Slight	37	71.2
Carriers	271	65.8
Total	433	69.2

Table V. Various situations giving rise to secondary cases

Source of infection	No.
Relatives or inmates of the home ill with diarrhoea	22
Care of persons ill with diarrhoea in the home	2
Fellow-workman ill with diarrhoea	1
Bacillary carriers at school	6
Bacillary carriers in the home	3
The mother bacillary carrier at delivery	1

Secondary cases

As was mentioned above 35 cases were regarded as secondary to other infected persons. Seventeen (48.6 %) were children below 16 years of age. Table V gives the characteristics of the various sources of infection. It should be noted that the infection in 25 cases seemed to derive from persons in the environment, who were ill with diarrhoea, while infection secondary to carriers in the home occurred only in three instances from two homes. Six school-children probably became infected secondarily to carriers in their classes. It seems reasonable to suppose that the spread of the infection in th schools was due to poor lavatory hygiene.

One baby whose mother was carrier was born in our hospital and found to be infected from birth. The clinical data were as follows.

Table II The distribution according to age of the total Uppsala material (groups A, B C, D) and of the material more thoroughly studied (groups A B C)

Age group (yrs)	Material A, B, C, D				Material A, B, C Total N = 626 (%)	
	Men N = 316 (%)	Women N = 357 (%)	Total N = 673 (%)			
< 1	0.6	2.5	1.7	27.4	1.5	28.8
1–6	7.9	7.9	7.9		8.3	
7–15	20.3	15.7	17.8		19.0	
16–24	19.6	21.3	20.5	72.6	19.0	71.2
25–59	44.6	44.8	44.7		44.7	
>60	7.0	7.8	7.4		7.5	

Table III The distribution of different grades of severity of illness within different age groups of the A B C material (N = 626)

Grades of illness	<1 yrs N = 9 (%)	1–6 yrs N = 52 (%)	7–15 yrs N = 119 (%)	16–24 yrs N = 119 (%)	25–59 yrs N = 280 (%)	>60 yrs N = 47 (%)
Severe	–	–	0.8	–	1.4	4.3
Moderate	(33.3)	28.8	11.8	26.1	25.7	42.5
Slight	(11.1)	5.8	4.2	10.9	8.2	14.9
Carriers	(55.6)	65.4	83.2	63.0	64.7	38.3

Distribution according to age

Table II shows the age distribution of the total Uppsala material and of the better defined material. There is no particular difference between the sexes apart from the age group 7–15 years, which has some preponderance of boys. This is probably due to selection of the material, perhaps a predominance of errand boys at the provision stores.

Distribution according to severity of infection

The material A, B C (N = 626) was divided into four groups according to the various degrees of illness.

1 Cases with *severe symptoms (1.1)* The patients of this group exhibited fever above 38° C, and had diarrhoea and/or vomiting In addition to these signs they showed prostration and developed serious symptoms of dehydration usually in combination with a markedly septic state with prolonged high fever One patient, an elderly man, died from septicaemia on the eleventh day of illness.

2. Cases with *moderately severe symptoms (24.8)* The patients of this group had fever above 38 C, diarrhoea and/or vomiting, but their condition was not critical. Although the disease did not take a serious turn, many of them were very ill, particularly in the beginning having high, septic fever and violent diarrhoea. On the other hand some of the patients in the group were only mildly ill.

3. Cases with *slight symptoms (8.3)* The patients of this group had diarrhoea and/or vomiting but no fever and their illness was very mild and usually of short duration.

4 *Bacillary carriers (65.8)* These patients denied any symptoms whatsoever but excreted S. Newport in faeces. A few patients with vague and non-specific symptoms were assigned to this group.

The distribution with regard to severity of infection was the same in both sexes. On the other hand a considerable difference in

Time to freedom from bacilli

Many patients had to bear long period of isolation either in hospital or at home. The following requirements for freedom from bacilli were laid down, in accordance with established practice in Sweden. The patient must have at least three negative faecal cultures in succession with an interval of at least three days between each specimen. One or tw further negative cultures were required for food handlers and medical personnel. The specimens were not to be taken during or in direct connexion with a course of treatment which could be expected to influence the result of the culture.

The length of the carrier state was defined as the time from the day of consumption of infected pastry to the day of the first negative faecal specimen, followed by t least two further negative samples. The faecal specimens for culture were obtained regularly from practically every patient.

It has been possible to calculat exactly the period of excretion according to the definitions just mentioned in 362 out of the 698 cases making up the groups A, B, C and E (51.9) Table VII presents the distribution of the 362 cases in three grades of illness and the cumulative percentages of patients free from bacilli within 3, 6, 9 12 and 15 weeks. It is evident from the table that the proportion of patients with negative specimens after any time is higher among the carriers than among the patients with severe or moderate symptoms. However the difference is marked only at three weeks, indicating that many were transient carriers only One single positive faecal specimen was observed in 77 instances among the 431 carriers in groups A, B, C and E (17 Some of the transient carriers were however examined some weeks after the period of risk and might naturally have had positive cultures prior to the examination. The patients with mild symptoms follow the carriers Seven of the 50 patients in groups A, B, C and E with mild symptoms (12 %) were transient carriers

Generally can be stated that half of the patients, independent of their degree of illness, were still excreting bacteria in the faeces after six weeks No relationship has been found between the length of the carrier state, on the one hand, and age sex or length of the incubation period, on the other

Table VI The incubation period of 161 patients related to the grade of illness (A, B C, E material)

Incubation period (days)	All cases N=161 (%)	Grade of illness		
		Severe N=7 (%)	Moderate N=125 (%)	Slight N=29 (%)
≦ 2	67 7	100.0	69.6	51.7
3—7	28.0	—	26.4	41 4
8—12	4.3	—	4.0	6.9

Table VII. The cumulative percentages of patients free from bacilli within 3, 6 9, 12 and 15 weeks (A, B C, E material)

Free from bacilli within (weeks)	All cases N=362 (%)	Grade of illness		
		Severe or moderate N=132 (%)	Slight N=36 (%)	Symptomless carriers N=194 (%)
3	12.2	4.5	19.4	16.0
6	48.9	41.7	50.0	53.6
9	84.5	73.0	92.0	89.7
12	93.9	90.2	100.0	99.0
15	98.3	96.2	—	99.5

Various antibiotics were administered to a number of patients during the period of excretion of bacilli, and the influence of this therapy must be taken into account. However the therapy given could not possibly have had any influence on the pattern demonstrated in table VII, because only 8.2 % of the carriers and 19 4 of the patients with slight symptoms were treated within the first three weeks, while as many as 31 % of the patients with severe or moderate symptoms received treatment. A certain effect of the therapy with regard to shortening of the period of excretion was noted within the three categories of table VII, however

As has been mentioned previously four or five negative faecal specimens in succession

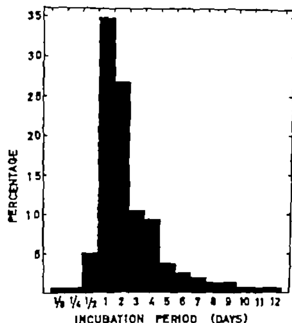


Fig. 2 The percentage distribution of the incubation period of 161 cases.

The mother a nurse 24-year-old, was pregnant with expected delivery on June 12, 1960 The pregnancy had been uncomplicated The mother was a customer at a shop, to which the bakery in question delivered provisions. On May 23 she was taken ill with fever vomiting and diarrhoea. The symptoms subsided after three days. S Newport was isolated from a faecal specimen taken on May 23 Her husband also had a positive culture on this day The mother was admitted in good health on May 29 A urinary culture was positive on May 30 Faecal cultures remained positive until June 18 The husband was negative as early as May 28.

The labour started on June 6 in the evening and eight hours later she gave birth to a healthy girl with a birth weight of 3 140 g The delivery was uneventful. An attempt to sterilize the intestinal content of the mother before the delivery with Enterobiotic® (neomycin + oxytetracycline) was unsuccessful because of the sudden delivery and the short labour A faecal specimen from the mother at birth and a specimen of the amniotic fluid were both positive. The child did not show any signs of disease and gained in weight satisfactorily the weight on discharge on June 26 being 3,660 g However S Newport was isolated from the faeces of the child for as long as 10 weeks (the last positive sample was obtained on Aug 13)

In all likelihood the baby girl contracted her infection during the birth process by swallowing contaminated amniotic fluid. Similar cases have been described repeatedly in the literature (6, 17 35) In some instances it has been presumed that the infection occurred during the intrauterine life (1 2). It is of interest that the baby excreted bacilli for so long a period as 10 weeks, long after the time when the parents had become negative.

Incubation period

It was possible to calculate the incubation period very accurately in a large number of instances, because many patients with symptoms knew exactly the day and hour of consumption of the infected pastry Such a calculation could be carried out in 161 out of 247 patients with symptoms (65.2) in the groups A, B, C, E (N = 698) It will be seen from fig 2 that the incubation period was one or two days in the majority of cases. An incubation period of 24 hours or less was found in 41 and of 48 hours or less in 67 7 The shortest incubation period observed was three hours, noted once in a very ill patient. On the whole, there seemed to be a tendency among the patients with severe or moderately severe illness to have a shorter incubation period than among those with milder illness, as appears from table VI This correlation most likely reflects the relationship between the dose of bacteria ingested and the reaction of the host, inasmuch as the swallowing of a large number of bacteria can be expected to enable a large number of virulent organisms to escape the acidity of the stomach and enter the intestines, rapidly giving rise to symptoms of disease.

An observation of clinical importance is that the incubation period in salmonella gastroenteritis due to S. Newport may be longer than is usually expected. Thus, 45 patients (28) said that the ingestion of the particular cakes occurred 3—7 days before the onset of symptoms, and seven patients (4 3) stated that the only consumption of infected cakes they were aware of occurred in fact 8—12 days before.

There was no difference in incubation period with regard to the different age groups.

Time to freedom from bacilli

Many patients had to bear long period of isolation either in hospital or at home. The following requirements for freedom from bacilli were laid down, in accordance with established practice in Sweden. The patient must have at least three negative faecal cultures in succession with an interval of at least three days between each specimen. One or tw further negative cultures were required for food handlers and medical personnel. The specimens were not to be taken during or in direct connection with a course of treatment which could be expected to influence the result of the culture.

The length of the carrier state was defined as the time from the day of consumption of infected pastry to the day of the first negative faecal specimen, followed by at least two further negative samples. The faecal specimens for culture were obtained regularly from practically every patient.

It has been possible to calculate exactly the period of excretion according to the definitions just mentioned in 362 out of the 698 cases making up the groups A, B, C and E (51.9 %). Table VII presents the distribution of the 362 cases in three grades of illness and the cumulative percentages of patients free from bacilli within 3, 6, 9 12 and 15 weeks. It is evident from the table that the proportion of patients w th negative specimens after any time is higher among the carriers than among the patients with severe or moderate symptoms. However the difference is marked only at three weeks, indicating that many were transient carriers only One single positive faecal specimen was observed in 77 instances among the 431 carriers in groups A, B, C and E (17 Some of the transient carriers were, however examined some weeks after the period of risk and might naturally have had positive cultures prior to the examination. The patients with mild symptoms follow the carriers Seven of the 59 patients in groups A, B, C and E with mild symptoms (12 %) were transient carriers.

Generally it can be stated that half of the patients, independent of their degree of illness, were still excreting bacteria in the faeces after six weeks No relationship has been found between the length of the carrier state, on the one hand, and age, sex or length of the incubation period, on the other

Table VI The incubation period of 161 patients related to the grade of illness (A, B C, E material)

Incubation period (days)	All cases N=161 (%)	Grade of Illness: Severe N=7 (%)	Moderate N=1.3 (%)	Slight N=29 (%)
≤ 2	67 7	100 0	69.6	51.7
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Free from bacilli within (weeks)	All cases N=362 (%)	Grade of illness: Severe or moderate N=132 (%)	Slight N=36 (%)	Symptomless carriers N=194 (%)
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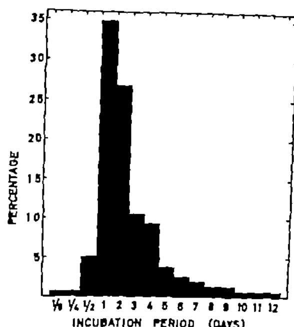


Fig 2 The percentage distribution of the in cubation period of 161 cases.

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In all likelihood the baby girl contracted her infection during the birth process by swallowing contaminated amniotic fluid. Similar cases have been described repeatedly in the literature (6, 17 35) In some instances it has been presumed that the infection occurred during the intrauterine life (1 2) It is of interest that the baby excreted bacilli for so long a period as 10 weeks, long after the time when the parents had become negative.

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It was possible to calculate the incubation period very accurately in a large number of instances, because many patients with symptoms knew exactly the day and hour of consumption of the infected pastry Such a calculation could be carried out in 161 out of 247 patients with symptoms (65.2) in the groups A, B, C, E (N = 698) It will be seen from fig 2 that the incubation period *was one or two days in the majority of cases.* An incubation period of 24 hours or less was found in 41 % and of 48 hours or less in 67 7 *The shortest incubation period* observed was three hours, noted once in a very ill patient. On the whole there seemed to be a tendency *among the patients* with severe or moderately severe illness to have a shorter incubation period than among those with milder illness, as appears from table VI This correlation most likely reflects the relationship between the dose of bacteria ingested and the reaction of the host, inasmuch as the swallowing of a large number of bacteria can be expected to enable a large number of virulent organisms to escape the acidity of the stomach and enter th intestines, rapidly giving rise to symptoms of disease.

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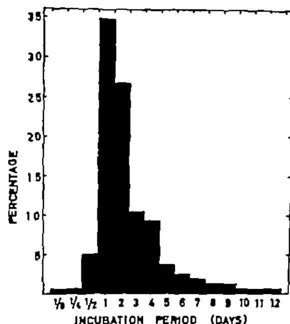


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It has already been pointed out that the salmonellae causing mainly enteritis may differ from type to type with regard to their tendency to invasiveness It is quite likely that they may also differ in other properties. A proneness to stay for a long time in the gastrointestinal tract may be a characteristic trait of some types, perhaps of members of group C The tendency to a prolonged incubation period in a substantial number of cases may be typical not only of S Newport but also of other types. Other characteristics may prevail To learn more about the disease patterns evoked by particular salmonella types it is essential that clinical and epidemiological studies of outbreaks should be made repeatedly

Summary

An extensive outbreak of food poisoning due to S Newport occurred in the Uppsala region in 1960 In this area 745 infected cases were known 698 of whom were studied in some detail.

Of 626 patients from Uppsala itself 69 2 per cent were known to have consumed heavily contaminated cakes from one large bakery and a further 16 1 per cent were customers in provision stores to which the bakery delivered its products. 5 6 per cent were regarded as secondary cases.

No less than 65.8 per cent of the patients from Uppsala were symptomless carriers only traced by comprehensive bacteriological investigations in the community 34 2 per cent had symptoms of varying severity Seven patients (1 1 per cent) were dangerously ill. There was one death from salmonella sepsis.

The incubation period of 161 patients was in the majority of cases less than 48 hours. It is noteworthy however that 28 per cent had an incubation period of 3—7 days and four per cent 8—12 days.

The period to freedom from bacilli in faeces was remarkably long Thus, after six weeks only 50 per cent of the patients were free of bacilli and after nine weeks 15 per cent were still excreters. The symptomless carriers were clear somewhat more rapidly than those with marked symptoms.

Most of the secondary cases derived from contact with patients ill with diarrhoea. Data are presented which argue in favour of isolation in the home of the comparatively harmless carrier Certain categories should however be isolated in the hospital

It is pointed out that it is important to analyse any major epidemic if we wish to learn the characteristic features of infection with a certain type of salmonella

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Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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Familial Hypercholesterolaemic Xanthomatosis and Coronary Disease

By

Josef Burstein and Carl W Malm

Familial hypercholesterolaemic xanthomatosis (FHX) or Muller Harbitz disease has long been the subject of clinical investigations especially in the U S A (2, 3 4 7 9 11 14 19 21 25 33 37) in Norway (13 16, 17 26 32) and in Denmark (22, 29) In Great Britain the first report was published in 1956 (23) From Finland there have been no reports so far The interest in this disorder has, however been maintained and even increased by the widely accepted belief in the central role played by a disturbed lipid metabolism, i. e. hypercholesterolaemia in coronary atherogenesis In fact, in many members of families with hypercholesterolaemic xanthomatosis an intimate relationship between the inborn error of lipid metabolism and the occurrence of coronary disease seems to be evident. The families involved are thus suitable objects for studying the interplay of different factors of possible significance in coronary atherogenesis and thorough mapping of these family groups is warranted. When allowance is made for the indistinct boundaries between FHX and essential hypercholesterolaemia, it may be that observations within the first group have some bearing on the second.

Setting out from the considerations mentioned this report concerns a rather large family group with hypercholesterolaemic xanthomatosis. Special attention has been paid to the occurrence of ischaemic heart disease with and without evident disturbance of lipid metabolism.

Material and methods

The family originates from the southeastern part of Finland, the great majority of its members still living in the same region and exhibiting a rather uniform socio-economic status and way of life. Data for four generations are available. The first generation is represented solely by an ancestor the late (No. 1) who according to report suffered from heart disease and exhibited xanthelasmata on his eyelids. The next or second generation comprises his eight children, four of whom are alive. The third generation includes 25 individuals, 21 of them still living. The fourth generation is represented by 12 children, all of them alive.

The investigation of the living members included ordinary physical examination noticing especially findings from the cardio-

Submitted for publication October 9 1963.

Table I Serum cholesterol, total lipids lipoprotein ratio, ECG and miscellaneous findings

No.	Age	Sex	Serum cholest. (mg%)	Total lipids (mg%)	Lipo-proteins (α/β γ)	ECG	Miscellaneous findings
1	?†	♂	—	—	—	—	Said to have had xanthelasmata and heart disease. Died of malignant tumour
2	61	♀	229	618	0.44	Normal	Systolic murmur grade II III max. in the aortic area
3	59	♀	496	939	0.30	S-T segment depressed 2 mm in II III VF V4—V6	Heart sounds dull, ectopic beats (?) by auscultation B.P. 220/130
4	51	♀	368	923	0.36	Normal	Tendinous xanthoma on the back of the left hand. Corneal arcus. Palpatory weak pulsation of a. dors. pedis l. a.
5	62†	o	—	—	—	—	Was fat, said to have had xanthelasmata. Died of a entricular malignancy
6	62†	♂	—	—	—	—	Was fat said to have had xanthelasmata. Died of heart disease
7	64	♂	332	700	0.43	Normal	Corneal arcus. In a. dors. pedis l. a. palpatory weak pulsation
8	52†	o	—	—	—	—	Said to have suffered from "asthma and t ha had xanthelasmata. Sudden cardiac death
9	<50†	o	—	—	—	—	Committed suicide
10	36	♂	158	564	0.33	Ventricular ectopic beats	—
11	26	♂	198	695	0.28	S1 S2, S3 pattern. QRS 0.11-0.12 sec.	—
12	bt. 20†	♂	—	—	—	—	Killed in action
13	29	♂	171	522	0.63	Normal	—
14	26	♀	169	541	0.81	Position vertical. Dextrogram. S-T segment depressed 0.5–1 mm in III VF biphasic T wave in III	

Fig 1 Pedigree of the family studied.

♂ ♀

■ ● Serum cholesterol ≥ 300 mg % 1st and 2nd, ≥ 250 mg % 3rd and 4th generation.

Deceased, hypercholesterolaemia and/or xanthomatosis probable according to the history

Deceased, hypercholesterolaemia and/or xanthomatosis improbable according to the history

□ ○ Normal serum cholesterol.

□ ○ Unexamined.

IHD = Ischaemic heart disease, D = Diabetes mellitus.

vascular system and the occurrence of tendinous or tuberous xanthomata, xanthelasma palpebrarium (xanthoma plana) and corneal arcus. The laboratory investigations comprised determination of serum cholesterol according to the method of Pearson et al (28) total lipids, lipoproteins electrophoretically (31) determination of sedimentation rate, haemoglobin, number of erythrocytes, and leukocytes, the prothrombin level, alkaline phosphatase and the protein reaction of Stolte The wife of a man belonging to the second generation (No 8) suffered from diabetes mellitus, in the children of this couple the glucose tolerance test was performed in addition to measurement of the blood sugar level. The ECG examination included the twelve lead electrocardiograph and V5 before and immediately one and three minutes respectively after exercise (knee-bending) In four individuals belonging to the third generation the ECG exhibited some changes, to be reported later on. In these cases the heart was examined roentgenologically in addition. All the laboratory tests, including ECG were performed in the fasting state The examination of the children of the fourth generation was incomplete because of the unwillingness of their parents to cooperate and was accordingly confined to the determination of serum cholesterol in four children only The laboratory tests of one woman (No. 34) were done in Sweden (Centrallasarettet, Linköping) In this case we have no information about the total lipids and lipoproteins. Another woman (No. 28) also living in Sweden refused the examination. The spouses of the second generation were examined, too, except the widow of No. 5. The examination of the spouses of the third generation was confined to determination of serum cholesterol in a few of them. The data concerning the deceased family members were based on interviews with relatives and on hospital records available in a few cases.

The upper limit of a normal serum cholesterol level was arbitrarily (because rigid demarcating criteria do not exist) fixed at 300 mg for the members of the second generation (51 – 64 years) and at 250 mg for those of the third and fourth generations (maximal age 34 years) Sex differences were not taken into account. The ratio of α-lipoproteins to the sum of β- and γ-lipoproteins was determined.

The inborn error was considered manifest if xanthomata or xanthelasmata were observed and/or the serum cholesterol level exceeded

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14	26	♀	169	541	0.81	Position vertical. Dextrogram. S-T segment depressed 0.5–1 mm in III VF biphasic T wave in III	–

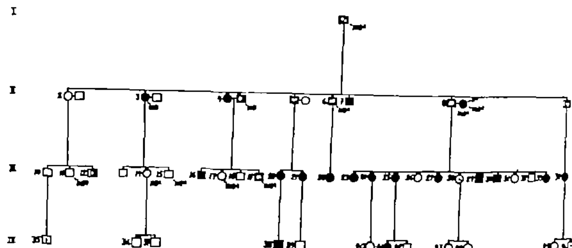


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Table 1 (cont.)

No.	Age	Sex	Serum cholest. (mg%)	Total lipids (mg%)	Lipo-proteins (α/β+γ)	ECG	Miscellaneous findings
31	18	♀	225	710	0.44	Normal	Fasting blood sugar 98 mg%. Glucose tolerance test 15 – 163 mg% 30' 218 mg% 4 hr–70 mg%. Mother diabetic
32	16	♂	223	529	0.35	Normal	–
33	14	♀	393	1,005	0.27	Normal	–
34	26	♀	269	–	–	Normal	Examination performed at the Linköping Hospital (Sweden)
35	5	○	–	–	–	–	–
36	4	○	–	–	–	–	–
37	1	○	–	–	–	–	–
38	9	○	367	–	–	–	–
39	1	○	–	–	–	–	–
40	6	♀	–	–	–	–	–
41	1½	♀	287	–	–	–	–
42	1 month	♂	172	–	–	–	–
43	4	♀	–	–	–	–	–
44	2	♀	–	–		–	–
45	Unknown	♀		–	–		–
46	Unknown	♀		–	–	–	–

the upper limit of normal. In the case of deceased individuals we used the same criteria based on data available from the history.

Results

The pedigree of the family is illustrated in fig. 1. In table I the results of our investigation are presented in detail.

Three of the four living members of the second generation exhibited hypercholesterolaemia (496, 368, 332 mg %) as well as some degree of hyperlipaemia (999, 923, 700 mg %). In one of the three cases clear-cut ischaemic ECG changes were observed (No. 3), in another (No. 4) tendinous xanthomata and corneal arcus, in the third (No. 7) corneal arcus only. In the last-mentioned two cases no cardiovascular abnormalities were found except for a somewhat weak peripheral pulsation in their feet. In the fourth normocholesterolaemic case (No. 2) a systolic murmur grade 2—3 was heard. According to the history of the four deceased members of the second generation, three of them had had

Table I (cont.)

No.	Age	Sex	Serum cholest. (mg%)	Total lipids (mg%)	Lipo-proteins (α:β+γ)	ECG	Miscellaneous findings
15	24	♂	209	631	0.50	Position vertical. Dextrogram. Nodal ectopic beats. S-T segment depressed 0.5–1 mm in III aVF	Heart sounds dull, moderate. Heart dilatation. Vol. 1 040/630
16	23	♂	357	913	0.37	Normal	—
17	17	♀	194	595	0.55	Ventricular ectopic beats. Insignificant depression of S-T segment in III aVF. Postexertional flat depression of S-T segment 0.5 mm in V5	—
18	15	♂	201	512	0.27	Normal	—
19	7†	♂	—	—	—	—	Said to have had fatty nodes on his legs and to have suffered from heart disease. Sudden cardiac death
20	34	♀	420	923	0.35	Normal	—
21	27	♀	310	65[illegible]	0.32	—	—
22	6†	♀	—	—	—	—	Said to have died in acute asphyxia. Her mother suffers from bronchial asthma
23	14†	♀	—	—	—	—	Died in diabetic coma
24	27	♀	360	799	0.44	Normal	—
25	26	♀	276	820	0.37	Normal	Exertional dyspnoea. Systolic murmur in the aortic area
26	24	♀	245	641	0.27	Normal	—
27	23	♀	363	1,020	0.21	Normal	Serum slightly lactescent. Tendinous xanthoma located on the second finger of the left hand. Cold feet. Palpatory week pulsation of a. dors. pedis on both sides
28	22	♀	—	—	—	—	Refused examination
29	21	[illegible]	412	966	0.28	Normal	—
30	19	[illegible]	475	1,077	0.18	Normal	Tuberous xanthomata on both knees, both elbows and right wrist

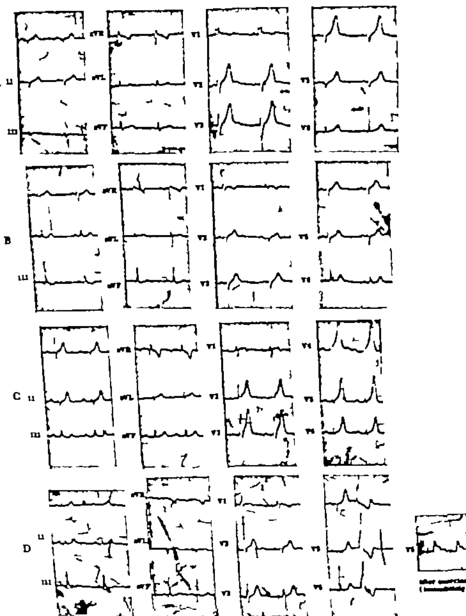


Fig. 2 ECGs of Nos. 11 (A) 14 (B) 15 (C) 17 (D) (see text or table I)

B. P. 130/90. X-ray of the heart normal. Serum cholesterol 198 mg. Total lipids 693 mg. Lipoprotein ratio 0.26. ECG (fig. 2 A) Heart rate 60/min. QRS complex widened 0.11 – 0.12 sec., S1, S2, S3 pattern, rSr/V1.

Case 14 Female, 26 years of age. Mother hypercholesterolaemic with clear-cut signs of ischaemic heart disease and essential hypertension, in addition possibly obliterative atherosclerosis in her legs. Father a spouse, normocholesterolaemic. History uneventful.

Table II Lipoprotein ratio in relation to age and sex Distribution of lipoproteins

2nd generation (♀)		2nd generation (♂)		3rd generation (♀)		3rd generation (♂)	
Case no.	α β + γ	Case no.	α β + γ	Case no.	α β + γ	Case no.	α β + γ
2	0.44	7	0.45	14	0.81	10	0.33
3	0.30			17	0.55	11	0.26
4	0.36			20	0.35	13	0.63
				21	0.32	15	0.30
				24	0.44	16	0.37
				25	0.37	18	0.27
				26	0.27	29	0.28
				27	0.21	30	0.18
				31	0.44	32	0.33
				33	0.27		
Average	0.37		0.45		0.40		0.35

xanthelasmata and two of these had cardiac disease. One of the two died suddenly at 52 (No 8) The cause of death in the remaining three cases was cardiovascular in one (No 6) cancer of the stomach (No 5) and suicide (No 9) respectively

Of the 25 members of the third generation four were deceased One girl died of asphyxia (?) at the age of 6 her mother a spouse suffers from bronchial asthma Diabetic coma was the cause of death in a girl aged 14 (No 23) In her mother also a spouse, mild diabetes mellitus was diagnosed a few years ago The third deceased member of this generation was killed in action when about 20 years old (No. 12) The fourth a boy of 7 died suddenly (No 19) He had been greatly invalided by heart disease of unknown origin and had had fatty nodes on his legs.

Hypercholesterolaemia was found in ten of the twenty tested members of the third generation Total lipids exceeded 700 mg % in all ten, the highest value amounting to 1 070 mg % In one case (No 27) the serum appeared slightly milky total lipids amounting to 1 020 mg % The ratio α-lipoproteins to the sum of β and γ-lipoproteins in the individual cases and the average values in relation to age (= generation) and sex are presented in table II In three cases (Nos. 26 27 33) the ratio fell below the lowest values found in healthy individuals (15) The average values for both sexes of the third generation were *definitely lower than those observed in a* series of healthy persons of corresponding age and sex (15)

Xanthomata were observed in two cases, tendinous in one (No 27) tuberous *in the other (No. 30) In both cases* hypercholesterolaemia (363 475 mg %) hyperlipaemia (1 020 1 077 mg) and a low lipoprotein ratio (0 21 0 18) were observed

Electrocardiographic anomalies were observed in four individuals belonging to the third generation The cases involved will be described in detail

Case 11 Male 26 years of age. Parents in good health, normocholesterolaemic. History uneventful Physical findings normal.

The widowed spouse of case No. 8 suffers from mild diabetes mellitus controlled by a dietary regimen alone. She exhibited moderate hypercholesterol aemia (321 mg %) possibly secondary to the diabetes.

The occurrence of sudden death in FHX is not rare and is regarded as typical (7). According to the history sudden death had occurred in two individuals belonging to our series (Nos. 8, 19). It is noteworthy that one of them was a boy of seven, who probably exhibited manifest xanthomatosis. Both his parents were probably hypercholesterolaemic.

In four cases belonging to the third generation (Nos. 11 14 15 17) we observed moderate or minor ECG changes preceding or after exercise. They have been presented above in detail. In some other cases a junctional S-T decrease was noted but not taken into account because of the lack of clinical significance of this anomaly (Åstrand (39) has, however recently pointed out the possibl significance of a junctional S-T decrease appearing at a slow heart rate.) The four individuals mentioned, two of them men, two women, were all young adolescents 17—26 years old. In none of them was there a history of rheumatic fever or other serious infectious conditions. In none was subjective heart trouble reported. In all of them the physical findings were unremarkable, except for dull heart sounds in one (No. 15). In the same case X-ray disclosed moderate heart dilatation. In all four cases the levels of serum cholesterol and total lipids, somewhat surprisingly were within the normal range, in one (N 11) the lipoprotein ratio was low but nevertheless within normal limits. Moderate and minor ECG changes, i. e. post-exertional S-T depression, in relatively young normocholesterolaemic members of families with hypercholesterolaemic xanthomatosis has been described before. Guravich's (14) series comprised 28 normocholesterol aemic individuals. In ten of them the ECG at rest was obtained, in six an additional two-step test was performed. In three individuals, aged 33 29 and 35 years respectively a positive response to the two-step test was noted (Nos. 36C, 7C, 10C) and in one of these symptoms suggestive of angina were observed in addition. The group with cholesterol levels ranging from 250—300 mg % and interpreted as "borderline" included 25 individuals. In 21 an ECG at rest was obtained, in 16 the two-step test was performed in addition. In four subjects under the age of 50 one of them possibly a spouse, post-exercise S-T depression (0.75—1.0 mm) was noted. The age of the positively responding persons, two of them women were 28, 37 38, 42 years respectively. In the large series of Epstein et al. (11) comprising 268 subjects, the ECG (at rest only) was obtained in most of them. In only a few were pathological changes observed, among them a normocholesterolaemic woman (No. 14) aged 34 who showed S-T depression of border line significance. In addition, two normocholesterolaemic subjects (Nos. 4 and 7) a sister and brother aged 14 and 8 years, showed partial heart block of unknown origin.

The total number of cases observed by us or mentioned by others exhibiting moderate or minor electrocardiographic anomalies in young or middle-aged individuals with normal or borderline serum cholesterol values amounted to fourteen six males and eight females, their ages ranging between 8 and 47 years. The number is small, but cardiovascular data concerning the normo-

No complaints. First sound split at apex. According to X ray heart volume within normal range. B. P 145/100 Serum cholesterol 169 mg % Total lipids 511 mg % Lipoprotein ratio 0 81 ECG (fig 2 B) Vertical position, dextrogram. Heart rate 55—60/min. S-T segment downward sloping in III and decreased 0.5—1 0 mm in III and aVF Biphasic T wave in III

Case 15. Male, 24 years of age. Brother of No 14 History uneventful. Heart sounds dull. B. P 140/90 X ray Moderate enlargement of the heart, volume 1 040/630 cm^3/m^2 Serum cholesterol 209 mg Total lipids 631 mg Lipoprotein ratio 0.50 ECG (fig 2 C) Vertical position dextrogram. Heart rate 75/min. Nodal ectopic beats prior to exertion and succeeding it. P-Q prolonged 0.26 sec. shortening immediately after exertion to 0 16—0 17 sec. S-T segment downward sloping in III and decreased 0 5 mm in III and aVF

Case 17 Female, 17 years of age Mother hypercholesterolaemic, showing tendinous xanthomata but no signs of cardiovascular abnormalities. Father a spouse, died at the age of 55 of myocardial infarction. In a paternal aunt the serum cholesterol is strongly elevated, she exhibits xanthelasmata and signs of ischaemic heart disease and peripheral obliterative atherosclerosis. A brother of the patient suffered from heart disease of unknown origin, as already mentioned. He died suddenly at the age of 7 and was said to have had fatty nodes on his legs. History uneventful Physical findings normal. B. P 145/100. X-ray of the heart normal. Serum cholesterol 194 mg / Total lipids 595 mg % Lipoprotein ratio 0.55 ECG (fig 2 D) Vertical position, normogram. Heart rate 55/min Occasional ventricular ectopic beats. S-T segment insignificantly decreased in III and aVF after exercise S-T segment decreased flat 0.5 mm in V5

The observations regarding the twelve children forming the fourth generation were confined to determinations of serum cholesterol in four of them two were found to be hypercholesterolaemic (Nos 38 41)

Comments

It is evident from the data presented that the family described typically represents familial hypercholesterolaemic xanthomatosis. Thus according to our criteria hypercholesterolaemia was present in 15 out of the 27 members of the family examined in the youngest at the age of eighteen months. The lipoprotein ratio showed a rough parallel with the cholesterol level In only three cases did the ratio fall below the limit values according to Hammarsten and Nilson's observations on a normal series (15) In a few individuals with normal or only slightly elevated serum cholesterol the ratio though within normal limits, fell considerably below the average values for healthy individuals of corresponding age and sex. It may be that the lowered lipoprotein ratio sometimes expresses more sensitively than the serum cholesterol level a disturbed lipid metabolism in FHX as Godal (13) has suggested A rough parallel between serum cholesterol and the level of total lipids was apparent. In one case (No 27) with total lipids amounting to 1 023 mg % the serum appeared slightly lactescent The lactescence of serum characterizing essential familial hyperlipaemia appears at considerably higher levels of total lipids beginning from 1,200 mg % The significance of the lactescent serum in this case remains obscure, but it should be borne in mind that FHX and familial hyperlipaemia have been observed in members of the same family (5)

The low incidence of xanthomata, observed in only three cases may be partly related to the predominance of relatively young people in our series Two of the individuals exhibiting xanthoma were only moderately hypercholesterolaemic (368 mg % age 51 and 365 mg age 23)

source of lipids in the formation of coronary atheromatosis in FHX not any more than is supposed to be the case in the pathogenesis of coronary atheromatosis in general (12 20) Hence it appears that coronary atheromatous may sometimes be a primary manifestation of the syndrome and not solely secondary to predisposing factors, as is generally believed. The basic metabolic events producing FHX are however still unknown

The boundaries between FHX and essential hypercholesterolaemia are indistinct, as are the boundaries between hypercholesterolaemia and normocholesterolaemia. Applying our observations to coronary atheromatosis in general, it seems that one of the keypoints of the problem of coronary atherogenesis constitutes the elucidation of the pathogenesis in subjects devoid of detectable deviations in lipid metabolism. In the clinical field investigations into the natural history of coronary disease in relatively young normocholesterolaemic subjects, including genetic information especially regarding possible links with the occurrence of essential hypercholesterolaemia and other lipidoses in their families, would possibly be useful contribution.

There are two main views regarding the mode of inheritance in FHX. Incomplete dominance has been proposed by Wilkinson et al. (37) and has found acceptance, for example by Adlersberg (3 4) and Hirschhorn et al. (19) According to this genetic model a single gene results in heterozygosis or the "forme fruste" of FHX, while homozygosis implies two alleles of the gene Homozygosis exists, according to Hirschhorn and Wilkinson (19) in the presence of xanthomatosis and/or hypercholesterolaemia exceeding 450 mg %. *In the opinion of* Piper and Orrild (29) Wheeler (36) Leonard (23) Harris-Jones et al (18) and others, the inborn error is inherited through a single dominant gene with incomplete penetration. None of the theories put forward has been definitely proved as pointed out by Epstein et al. (11) in their thorough consideration. Considering the possibility of ischaemic heart disease sometimes constituting a primary manifestation of the syndrome, it may be of interest to link the above reported cases with normal plasma lipids and suggestive incipient ischaemic heart disease in the genetic models outlined above. With regard to Nos. 14 15 and 17 (compare fig 1 and table I) the mode of inheritance in these cases seems to fit well with either of the prevailing theories. The parents of No. 17 are both afflicted, the mother fulfilling the conditions for homozygosis. Of their four children one died suddenly at the age of seven. He was known to suffer from heart disease and had fatty nodes on his legs. Another is hypercholesterolaemic, the *remaining two* (including No. 17) exhibiting normal plasma lipids. The mother of Nos 14 and 15 fulfils the requirements of homozygosis, all her *three* children showing normal lipid patterns so far The parents of No. 11 are both normocholesterolaemic. The aged father a spouse has no symptoms of cardiovascular disease except right bundl branch block. Of the three children one was killed in action as a young man, the two surviving sons both being normocholesterolaemic. The mod of inheritance is thus obscur in this case. Perhaps the ECG changes observed (S1 S2 S3 pattern broadened QRS complex, rSr/V1) are genetically connected with the RBBB of his father and completely unrelated to his membership of a FHX family

cholesterolaemic members of families afflicted with FHX are usually sparse and sometimes entirely lacking in reports dealing with this disorder

There are divergent opinions about the clinical significance of ECG changes of a degree observed in the cases presented by us as well as in the ones of Guravich (14) and Epstein et al (11) According to the recent studies of Mattingly (24) Rumball and Acheson (30) and Åstrand (39) even minor ECG changes (i. e. flat or sagging S-T depression of at least 0 5 mm) are suggestive of ischaemic heart disease or may signal a higher than average risk of developing *manifest* coronary disease. Thus it seems unjustified to ignore the possible significance of the ECG changes described by us and others. In our opinion the presence of latent ischaemic heart disease in at least some of these cases seems probable. Of interest in this connection is of course *the apparently normal lipid pattern* (determination of the triglyceride fraction was not performed however) or at least normal or borderline serum cholesterol level. Coronary atheromatosis is a common finding in young people in the western countries, as evidenced for example by the findings of Enos et al (10) and Osborne (27) and is by no means rare in normocholesterolaemic men (6 8) In the series of Adlersberg et al. (2) for example, 49 of 122 coronary patients below the age of 50 showed a cholesterol level of less than 300 mg % Ischaemic heart disease even in young normocholesterolaemic members of a FHX family may thus perfectly well occur by chance. Another alternative deserves consideration in as much as the postulated incipient *coronary* disease in these individuals may be a primary manifestation of the inborn error prevalent in their families. Normocholesterolaemia does not rule out the presence of FHX, according to a statement of Engelberg and Newman (9) "The blood cholesterol is usually elevated in this syndrome but not necessarily so. The three cardinal signs — hypercholesterolaemia skin or tendon xanthomata and coronary insufficiency — frequently occur together but any one or two of the triad may be absent or precede the other This seems to hold true even for the occurrence of xanthomata in normocholesterolaemic subjects (1 35) Some of the cases mentioned by us may be *monosymptomatic*, the inborn error of lipid metabolism manifesting itself at least so far by latent ischaemic heart disease only The extraordinarily young age of the subjects eight out of fourteen being under 30 and only one over 40 (in addition the literature mentions about a few aged members of FHX families *presenting coronary* disease and normocholesterolaemia) and the sex distribution eight females against six males, may support this view

It seems, then that coronary disease may arise in relatively young members of FHX families with small or entirely undetectable changes in their plasma lipid pattern The metabolic disorder may thus involve not only the homeostatic regulation of cholesterol as proposed by Williams (38) but in addition the metabolic events locally in the arterial wall (and the skin?) as well perhaps at a cellular level and perhaps similar to the mechanism operating in "normocholesterolaemic xanthomatosis (Hand-Schüller-Christian disease, Letterer-Siwe disease eosinophilic granuloma) according to Thannhauser (34) If true it seems improbable that filtration of lipids from the blood represents the only

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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Serous Meningitis

A Catamnestic Study

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Since Wallgren (31) in 1924 described the syndrome, acute aseptic meningitis, entirely on clinical basis, virological research methods have improved steadily so that one is now able, in an increasing number of cases, to establish the aetiology of this disease. It is a question in the first place of isolating the virus and demonstrating the presence of virus antibodies in the serum The virus types most often demonstrated in this connection in Denmark are in addition to parotitis virus the so-called entero virus, of which up until the present 3 polio types, 30 coxsackie types, and 29 ECHO types have been isolated, although all these have not been proved pathogenic to man let alone neurotropic (22, 28) Occasionally the following can be the causative organism Herpes simplex, influenza, adenovirus and zoster also varicella, morbilli, mononucleosis, rubella and leptospirosis (18, 23) In other countries, amongst others U.S.A. 13) and England (11) lymphocytic chorio-meningitis is a common aetiology and in East and Central Europe, also lately in Finland (19 20) and Sweden (7 8) the insect born virus of group RSSE is found, which is becoming more frequen as the cause of neuroinfections with or without paresis.

Owing to the fact that the problems o aetiology have not been fully elucidated and owing to the gradual passage be tween th purely meningeal symptom and the more parenchymatic affection the terminology of infectious diseases o the central nervous systems is somewha loose (10 18) In selecting the presen material serous meningitis has been de fined as being predominantly lymphocyt meningitis, presumably caused by eith virus or leptospira, with sudden onse fever headache, catarrh, muscle pain t gether with meningeal symptoms, such stiffness of the neck and back, buth wit out well defined symptoms resulting fro cerebral or spinal involvement. Paraly polio, encephalitis and polyradiculi have not been included.

Catamnestic studies of serous meningi have been carried out earlier (5 14) b

Submitted for publication October 9 1963.

Summary

A family of familial hypercholesterolaemic xanthomatosis (FHX) is presented. Out of twenty living members of the third generation who underwent investigation four aged 17 24 24 and 26 years respectively showed minor electrocardiographic changes compatible with ischaemic heart disease. In all the plasma lipid pattern was within normal limits. In the literature there are further reports of a few cases with normal or borderline serum cholesterol values showing electrocardiographic changes i.e. a positive response to the two-step test. The possible importance of the findings is stressed. It seems that the metabolic disorder in FHX may involve not merely the homeostatic regulation of cholesterol but also metabolic events occurring locally in the arterial wall (and the skin?) at a cellular level

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accredited to their neuroinfection. Two had endocrine disturbances, 2 epilepsy 2 tonic pupils, 2 paroxysmal orthostatic giddiness, 13 headache, and 20 mental disturbances. Four of the last group were handicapped with regard to their working ability as result of this disability

Benediktsson et al. (1) found after a written enquiry to 29 patients admitted to hospital during a meningitis epidemic caused by ECHO-9-virus no permanent disability in any of the patients.

Gregersen (5) after a written enquiry to 313 patients admitted to hospital suffering from primary or secondary lymphatic meningitis, found lasting complications in 89 patients (28 %) inasmuch as 4 had paralysis, 3 convulsions, 5 deafness, 32 headache, 25 giddiness, 9 reduced mental capacity 17 optic disturbances, 17 muscle pains, 25 neurasthenia and 1 St. Vitus Dance

Present study

At the medical dept. C, Odense Town and County Hospital, where the major number of infections of the central nervous system are admitted from the county during the four year period 1958—1961 83 patients were admitted suffering from serous meningitis.

There was no primary mortality in the material and all the patients were found alive six months to 4 years after admission, at personal interview with the patients and their families In this interview an attempt was made to elucidate the possible psychical, neurological and endocrinal disturbances. They were questioned regarding their social status and adjustment either in school or place of work. After the interview somatic examination was carried out with special regard to neurological debilitations In addition information was obtained on later admissions, special examinations, also from ocational guidance centres and the family doctors. Tw patients, however were only contacted by correspond ence and telephone, owing to the fact that they had moved outside the county

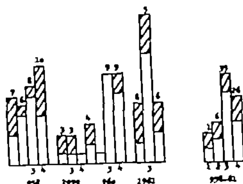


Fig 1 Seasonal variation (3 month period)
Aetiology known,
☐ Aetiology unknown.

I the above mentioned four year period the patients with a presumed infection of the central nervous system were examined with view to the possibility of virus being the causative agent, partly serologically and partly by sending samples of the faeces and cerebrospinal fluid to the State Serum Institute for examination for poliovirus and ECHO-virus, also in number of cases for virus of the coxsackie type. Thanks especially to this virus isolation t was possible to ascertain the aetiology in 34 of the cases, the distribution can be seen in table I Age and sex distribution are shown in table II It can be seen from this that in the group, under 20, the aetiology has been confirmed in 42 % of the cases, whilst it is only possible in 21 of those above 20. Further more the sex distribution is 1 1 for those patients without an aetiologic diagnosis, whilst in the group where the aetiology has been established it is possible to see the well-known predominance of male patients.

From fig 1 can be seen the seasonal variations with the greatest frequency in the lat summer and autumn, this is far more noticeable in the group of patients with unknown aetiology The cases of parotitis appear evenly distributed whilst enterovirus was found t be spasmodic, with regard to ECHO-9-virus it could be seen not only to be spasmodic but appeared to be geographically limited in several cases, where it was possible to prove infection from person to person.

I is not possible to find any definite variation within the various aetiological groups

Table I The aetiology of serous meningitis as shown from various studies

	Own study 1958–61	Quaade & Krogsgaard (21)	Just & Berger (9)	Oker Blom et al. (17)	Macrae (11)
No aetiology	52	72	–	65	–
Unidentified virus	6	23	–	15	–
Total	58 (66%)	95 (62%)	37 (67%)	80 (60%)	254 (77%)
Influenza	1	1	–	–	–
Adenovirus	1	1	–	–	2
Zoster	1	–	–	–	–
ECHO-9	8	–	3	–	–
Coxsackie B-2	5	1	–	}	}
Coxsackie B-5	2	23	–	} 1	} 3
Coxsackie A 1	–	2	–	}	–
Polio 1	5	–	}	}	60
Polio 3	1	–	} 8	} 36	–
Herpes simplex	–	–	–	1	1
Leptospirosis	–	–	–	3	2
Mononucleosis	–	1	–	–	–
Varicella	–	1	–	–	–
Morbilli	–	5	–	–	–
Parotitis	6	23	7	11	8
Total	30 (34%)	58 (38%)	18 (33%)	54 (40%)	76 (23%)

Table II Age and sex distribution from own study

	Age					♀	♂	Total
	0–5	5–10	10–20	20–50	50–			
No aetiology	6	11	10	24	1	27	25	52
Unidentified virus	–	4	1	1	–	2	4	6
A-influenza	–	–	–	1	–	–	1	1
Adenovirus	–	–	–	1	–	–	1	1
Zoster	–	–	–	1	–	–	1	1
ECHO-9	1	3	2	2	–	3	5	8
Coxsackie B-2	–	3	2	–	–	3		5
Coxsackie B-5	–	1	–	1	–	–	2	2
Polio 1	–	5	–	–	–	1	4	5
Polio 3	–	–	–	1	–	1	–	1
Parotitis	1	2	3	–	–	1	5	6
Total	8	29	18	32	1	38	50	88

only a few in which due regard has been given to the aetiological factors.

Müller and Nylander (14) found when examining 238 patients who had recovered from primary lymphocytic meningo-encephalitis, of which 138 were paired with controls 32 patients (13%) with permanent disability which could be

melaena. In three patients, all children, there appeared directly after the illness a marked increase in weight which was evidently caused by increased appetite, and which ceased approximately six months later. The adiposity in these patients was not obvious but was uniformly distributed in the subcutaneous fat. One of these patients had been admitted to hospital owing to his fatness without any signs of endocrine disturbances being found and the weight was reduced by reduction in calorie intake. At the control examination all of these patients were only slightly adipose.

In 13 patients permanent sequelae were found, which could be ascribed to the earlier serous meningitis. Their distribution according to aetiology and age can be seen in table IV. Also here the earlier mentioned mental after effects dominate. Three children showed moderately retarded development although this did not result in invalidation. One of these had in addition symptoms similar to petit mal but had normal electroencephalogram. One child had developed nocturnal enuresis. Only one patient was considerably handicapped after meningitis caused by A-influenza virus.

45-year-old farmer admitted 3.4.–16. 5. 1959. 9-year-old cranial injury and 17-year-old pleurisy. Otherwise healthy and able-bodied. A week prior to admission febrile with pain on swallowing together with soreness radiating to both sides of the neck. Afebrile after 4 days without specific treatment. One day prior to admission high temperature, headache, nausea and vomiting. Examination on admission showed stiffness of the neck and back but otherwise no neurological abnormalities. The temperature returned to normal within a week but the patient had to remain in bed for 5 weeks, mainly owing to headache and giddiness. These after effects persisted to some degree on discharge.

Laboratory examinations. CSF contained 710 leucocytes per mm³ protein 108 mg%, glucose 55 mg% pressure 120 mm of water. Bacteriological examination of the CSF. No growth of bacteria or virus. Meningococcal complement fixation test, parotitis complement fixation test and polio complement fixation test negative. Influenza complement fixation test A 4–64. Remaining influenza complement fixation tests negative. Electroencephalogram showed nothing definitely abnormal.

After discharge the patient was still unable to work owing to lassitude, headache, uncharacteristic giddiness, reduced powers of concentration and partial amnesia. This resulted in the patient having to sell his farm. In November 1960 the patient was admitted to the neurological department but on examination no signs of organic nerve disease could be found and a diagnosis of secondary hysterical neurosis and neurolabyrinthopathia toxica was made. The patient has since attempted to take lighter work unsuccessfully inasmuch as he still complains of lassitude uncharacteristic giddiness and reduced powers of concentration.

The 3 pregnant women in the material completed their pregnancy and gave birth to normal full-born children who on control-examination were found to be normal and well. The patient with the earlier paresis of the right lower extremity was symptom free and it was impossible to find neurological after effects in others.

One can thus see only a few (15) moderate after effects mainly of the mental type after serous meningitis. A comparison between the age groups show that the late prognosis is independent of age. Of the various aetiological groups it can be seen that parotitis meningitis is practically free from after effects, whilst half of the ECHO-9 infections included in this material resulted in permanent disability.

Discussion

The number of cases in which the aetiology has been elucidated in serous meningitis in the present material is found in table I and it can be seen from this that the level is comparable with that found in corresponding studies (9 11 17 21). A few authors find, however a somewhat greater frequency especially during epidemics (12 13 32). The influence of epidemics can clearly be seen from the studies mentioned in table I especially for those carried out over a shorter period.

The age distribution shows as in the case with Gregersen (5) a predominance of patients under 20 years of ge, whilst Muller et al. (14) found the reverse. That

Table III Temporary after effects (3—6 months) in relation to aetiology and age

	Total	Head-ache	Lassitude	Partial amnesia	Noise hyper sensity	Irrita-bility	Back ache	Nervous manifes-tations	Weight increase
No aetiology	12/52	4	8	2	1	2	—	—	2
Parotitis	1/6	—	—	—	—	—	1	—	—
Zoster	1/1	1	—	—	—	—	—	—	—
ECHO 9	1/8	1	1	—	—	1	—	—	—
Coxsackie B-2	2/5	2	—	—	—	—	—	—	1
Polio 1	2/5	1	—	—	—	—	—	1	—
0—5 years	0/8	—	—	—	—	—	—	—	—
5—10 years	8/29	5	2	—	—	1	—	1	2
10—20 years	2/18	1	—	—	—	—	1	—	1
20—50 years	9/32	3	7	2	1	2	—	—	—
Total	19/88	9	9	2	1	3	1	1	3

Table IV Permanent sequelae in relation to aetiology and age

	Total	Headache	Lassitude	Lack of concentration	Partial amnesia	Irritability	Giddiness	Increased sleep requirement	Backache	Enuresis	Petit mal	Nervous manifestations	Retarded development
No aetiology	5/52	3	3	2	—	—	—	1	1	1	—	1	1
Unidentified virus	2/6	1	1	1	1	—	—	—	—	—	—	—	—
A-influenza	1/1	1	1	1	1	—	1	—	—	—	—	—	—
ECHO-9	4/8	2	—	—	—	1	—	—	—	—	1	1	2
Polio 3	1/1	—	—	—	—	—	—	—	—		—	1	—
0—5 years	1/8	—	—	—	—	—	—	—	—	—	1	—	1
5—10 years	4/29	2	—	—	—	1	—	—	—	1	—	—	1
10—20 years	2/18	1	1	1	—	—	—	1	1	—	—		1
20—50 years	6/32	4	4	3	2	—	1	—	—	—	—	3	—
Total	13/88	7	5	4	2	1	1	1	1	1	1	3	3

with regard to the clinical course the CSF examination or the laboratory tests as a whole Sixteen patients had a dyphasic temperature chart without this being demonstrated as being characteristic for any one group One patient had a slight exanthema and a 2 year-old boy slight paresis of the right lower extremity in both of these cases it was impossible to give an aetiological diagnosis. Amongst the patients admitted there also were, in the group without a known aetiology 3 pregnant women in the 2nd 3rd and 5th month of pregnancy

In the control examination a characteristic finding was that the majority with slight emphasis on adults, had up to several weeks convalescence with moderate manifestations in the form of headache, lassitude bad memory noise hypersensity irritability and nervous manifestations as a whole.

In 19 patients (table III) there were found noticeable after effects, these varied from 3 to 6 months in duration but were however transitory These were on the whole an accentuation of the above post infective neu-

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it is possible to elucidate the aetiology in double the number of cases in children and young adults as in the higher age groups has not been found to have been mentioned in other studies

A predominance of male patients suffering from serous meningitis is a well known fact This predominance can be seen in the present material only in the cases where the aetiological factor has been determined. This fact can also be seen but to a lesser degree in the material collected at Blegdamshospital Copenhagen (21)

Seasonal variations show as in other works an increase in the number of cases in the late summer and autumn with epidemic like appearance of entero virus (6 30)

The 3 catamnestic studies mentioned earlier (1 5 14) and the present material cannot be directly compared owing to the above mentioned uncertain terminology and the somewhat varying methods of selecting the patients. Müller et al (14) found though 13 % with permanent after effects which agrees well with the 15 % found here. In addition the character of the after effects is also similar in type inasmuch as they are moderate disturbances of the mental faculties in fact only 1—2 % are actually handicapped Gregersen (5) found 28 % with sequelae, this often showed evidence of damage to the cerebral parenchyma whilst Benediktsson et al (1) found no after effects in 29 patients with ECHO-9 virus meningitis against the 50 % found in this material

There are thus serious shortcomings in elucidating the aetiology of serous meningitis which has in this material shown an extremely good prognosis. One has not in this material found virus types which from experience, often give permanent disability as for example coxsackie A 7 virus which can give a picture similar to polio (29) and arbor virus, which has up until the present only been seen, to any extent in this country on Bornholm (3)

Summary

In medical department C, Odense Town and County Hospital in the 4 year period 1958—61 88 patients with serous meningitis were admitted. Virus was found to be the infective agent in 30 of these cases (34 %) viz. in 21 male and 9 female patients. The aetiological elucidated cases were double the number in children than in adults, slightly more frequent in men than in women and comparatively seldom in the late summer and autumn.

The mortality was zero All the patients were control examined and all were found to be completely rehabilitated with the exception of one patient who had suffered from A influenza meningitis. Nineteen patients (22 %) had protracted but temporary after effects, mainly psychical 3 children had however pronounced increase in weight Thirteen patients (15%) had permanent disability of which 3 had moderately retarded development 1 with symptoms similar to petit mal and 1 with nocturnal enuresis. The other after effects were headache and lassitude together with psychical complaints in one case so pronounced that the patient was actually handicapped with regard to employment

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Aneurysms of the Coronary Arteries

Report of Two Cases

By

S. Bertelsen and A. Lindahl

Acquired lesions in the coronary arteries are of the greatest importance clinically and pathologically they concern primarily the atherosclerotic and/or thrombotic vascular changes.

Until a few years ago congenital abnormalities and anatomical anomalies of the coronary arteries were considered almost unique lesions, which were solely of academic interest and which were first recognized at autopsy. During recent years, however several cases of congenital coronary aneurysms have been reported of which few have had fistulas to the heart or to the great veins. A survey of the congenital aneurysms of the coronary arteries and of their possible treatment is published by Grob and Kolb in 1959 (8).

It is generally believed that Bougon (3) in 1812 was the first to report an aneurysm of a coronary artery but already in 1761 Morgagni (12) described a case with diffuse dilatation of the left coronary artery in a patient suffering from syphilitic aortitis. Several synopses of the published reports have appeard the first time being in 1929 by Packard and Wechsler (14) later reviewed and supplemented by Crocker et al. (5) Gore et al. (7) Rigdon and Vandergriff (16) and Scott (18). In Denmark Sondergaard (22) described the surgical correction of an aneurysm of the left coronary artery in a child, and Munkner et al. (13) are the first to have established the diagnosis by roentgenography.

To this day approximately 70 cases of coronary aneurysms have been reported. A little more than half of these are considered congenital or atherosclerotic aneurysms while the rest mostly are considered mycotic-embolic, syphilitic or rheumatic aneurysms, aetiologies which undoubtedly are of less significance today.

Two cases of coronary aneurysms with different aetiologies are described below. Both were found within a period of 6 months.

Submitted for publication October 10 1963.

dition improved considerably. During these days the temperature fell from approximately 40° C to 37.2° and jaundice decreased markedly.

Seven days after admission the patient vomited after eating grapes. Collapse occurred immediately after this, and despite artificial respiration she died soon afterwards.

Autopsy. The body was that of an 8-year-old fragile emaciated girl.

The principal interest concerned the heart. The pericardium contained about 200 ml partly clotted blood lying as a mantle around the heart. The pericardial surfaces were normal with no fibrinous exudate. The heart weighed 125 g and measured 10 × 9 cm, the left ventricular wall measured 10 mm, the right 3 mm. No congenital lesions of the heart or of the large vessels could be demonstrated. The foramen ovale and the ductus arteriosus were closed. The entire endocardium was normal, in particular no signs of ulcerative or vegetative endocarditis could be demonstrated. The valves were normal, and the myocardium was brownish-red with no signs of myocardial infarct.

The coronary orifices were normal, but in the left coronary artery however a cherry-sized, fusiform aneurysm with very thin and smooth walls without thrombi was found immediately after the origin from the aorta. Towards the pericardial surface a rupture approximately 1.5 mm in diameter was revealed (fig. 1). The remaining part of the artery was normal. In the right coronary artery about 2 cm from the aorta a green pea-sized, fusiform aneurysm of the same appearance as that on the left side was found. No rupture was found here, and the rest of the right coronary artery was also completely normal (fig. 2).

The coronary veins were normal.

The lungs were moderately oedematous and congested, and the lower part of both lobes were deflated with reduced amount of air. No foreign bodies were found in the respiratory tract.

The liver was slightly distended with yellowish-brown smooth surface, and the consistency was normal. The cut surface presented evident vascular structure. The gallbladder was slightly distended about the size of a goose-egg, and was thin walled containing copious amounts of clear watery mucus. The mucosa was slightly injected. The cystic and common ducts were normal. No concrements were found.



Fig. 3. Case 1. The left coronary artery just beyond the aneurysm. The tunica intima and the tunica media are infiltrated with polymorphonuclear leukocytes, and there is extensive necrosis of the media (hematoxylin-eosin, magnification × 30).

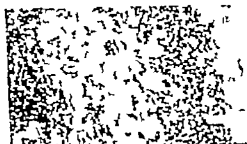


Fig. 4. Case 1. The right coronary aneurysm. The same microscopical picture as in fig. 3. There is extensive necrosis in all the coats of the vessel (hematoxylin-eosin, magnification × 120).

Microscopical examination of the arteries disclosed that the vessels were completely normal outside the aneurysms with regard to thickness as well as to stratification. Corresponding to the aneurysms pronounced changes of the wall were encountered in their entire extension. The tunica adventitia and adjacent subepicardial fat tissue were heavily infiltrated with neutrophil leukocytes, some lymphocytes, and a few eosinophil cells. The tunica media was markedly oedematous and packed with the above mentioned cells. The tunica intima showed marked proliferation with some granulocytic infiltration but mural thrombi were not seen. The entire aneurysmal wall was destroyed in several places to such an extent that it was impossible to distinguish the individual strata. No bacteria were seen in the vessel



Fig. 1. Case 1. An 8-year-old girl. Gross appearance of the left coronary artery, showing the aneurysm opened from the front. The stick is in the rupture.



Fig. 2. Case 1. Gross appearance of the right coronary artery, showing the aneurysm opened from the front.

Case reports

Case 1. 8-year-old girl who earlier had been well apart from bilateral acute otitis media at the age of 2 years. Routine prophylactical vaccinations had been done.

During the 6 months prior to admission the patient had scarlatina and oral penicillin was administered. Shortly after this pertussis was contracted, and the patient was treated with oral chloramphenicol. She did not quite recover from this last disease, attacks of high fever lasting a few days occurred with poor appetite and periodic vomiting. Continuous loss of weight was noted during the last month.

Four days prior to admission the girl was suddenly taken ill with high fever, distended glands on the neck and complaints of abdominal pain. The following days jaundice appeared. The stools were reported to be of normal shape and colour, and choluria was not observed. During the entire course of disease a dry cough was heard.

Physical examination. On admission the patient was rather poorly nourished, developed according to age. The temperature was considerably elevated, and she was acutely ill, dehydrated, with quite pronounced jaundice. There was stiffness of neck and back, but no Kernig's symptom. The pupils were normal but there was haemorrhagic conjunctivitis on both sides. The pharynx was diffusely red, and the posterior wall was oedematous and covered with thick mucus. The tonsils were normal. Along both sternocleidomastoid muscles several slightly tender, quite solid lymph nodes were palpated but apart from minor axillary nodes no further peripheral adenitis was seen. Examination of the chest disclosed normal limits of the heart, regular heart rhythm about 120. There was no pulse deficit and no definite murmur was audible. Pulmonary examination showed normal conditions. The abdomen was diffusely tender, slightly meteoristic but with no palpable tumors. Neurological examination was negative. There was no sign of heamorrhagic diathesis.

Laboratory tests. Examination of the blood revealed a Hb concentration of 74 and an ESR of 170 mm/hour. The white-cell count was 37 300/mm³ and the cell picture was normal. Serological tests for leptospira ictero-haemorrhagiae were negative and Bunnell's test was 1:16. Blood cultures showed no growth of bacteria. Chest roentgenogram and electrocardiography were normal. The urine was normal.

Course of disease. Due to a suggestive history of Weil's disease injections of terramycine and delcortine were commenced. Furthermore blood transfusions, fluids, and electrolytes were given intravenously, after which the con-

dition improved considerably. During these days the temperature fell from approximately 40° C to 37.2° and jaundice decreased markedly.

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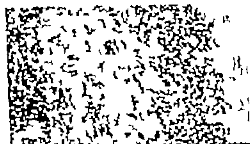


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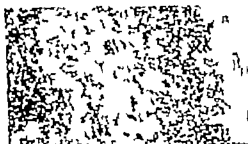


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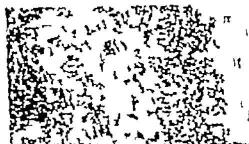


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Fig 5 Case 2 An 81 year-old male. The right coronary artery just beyond the aneurysm. There is vigorous proliferation and sclerosis of the tunica intima, and calcium salts are deposited (hematoxylin-eosin, magnification × 30)

walls. The distinct demarcation between the involved and the normal tissue was remarkable (figs. 3 4)

The entire myocardium was normal Its vessels were unaffected and there were no signs of rheumatic infection or other inflammatory changes.

Microscopy of the lungs, the kidneys, and the spleen disclosed only normal conditions. In the liver slight inflammatory processes were seen interlobularly in the periportal areas. The liver cells were normal. The adrenal glands were not microscopically examined The gall bladder presented slight, acute inflammation in all strata but with no microscopically visible vascular changes.

Case 2. The patient was an 81 year-old male with rapidly advancing strangury Before that he was well without cardiac symptoms.

Physical examination. Well preserved, lean man. Examination of the chest disclosed a slight dilatation of the heart. The heart rhythm was regular without pulse deficit. A systolic murmur was audible over the entire heart with maximal intensity at the apex. Pulmonary examination revealed normal conditions. Rectal exploration disclosed considerably enlarged prostate with several solid, nodular areas behind.

Laboratory tests. The Hb concentration was 86 % and the ESR was 37 mm/hour Serological test for syphilis was negative. The alkaline phosphatase was 5.4 units, and the acid phosphatase was 3 0 units. The serum creatinine was 3 6 mg/100 ml. The blood pressure was normal. Electrocardiography showed myocardial degeneration. Chest roentgenogram revealed normal heart shadow

Course of disease Transurethral prostatic resection was performed, and microscopy revealed carcinoma of the prostate. The patient was discharged on oestrogens but re admitted only a few months later on account of recurring difficulties of urination. Progressive renal failure occurred, and he died soon after in uraemia.

Autopsy The body was that of an 81-year old undernourished man.

The pericardium was normal with no accumulation of fluid or fibrin. The heart weighed 340 g and measured 11 × 12 cm, the left ventricular wall measured 14 mm, the right wall 4 mm. The endocardium and the valves were normal. Cut surface of the myocardium presented moderate fibrosis. The coronary orifices were normal. The coronary arteries were both markedly atherosclerotic. In the right coronary artery 2—3 cm from its origin a non-thrombotic saccular aneurysm approximately 2 cm long and 1 cm in diameter was seen. The endocardium was normal without signs of endocarditis, and the valves were normal. The myocardium showed chronic interstitial myocarditis but no signs of infarct.

The rest of the autopsy revealed striking aortic atherosclerosis. The markedly enlarged prostatic lobes projected up into the bottom of the bladder The kidneys were symmetrical, the surface being covered with fine granules. The cut surface of the cortex which showed atrophy measured about 4—6 mm in width. The pelves were slightly dilated and injected. Furthermore, metastases to the liver the spine and along the abdominal lymph nodes were found The lungs were moderately emphysematous with confluent bronchopneumonia.

Microscopical examination of the coronary arteries outside the aneurysm revealed considerable proliferation of the cells, and intimal thickening with precipitation of calcium salts in areas among the collagen fibres. The tunica media chiefly revealed normal stratification with longitudinal and transverse smooth musculature. Corresponding to the aneurysm the entire vascular wall was thinner and the tunica intima contained here abundant amounts

f calcium salt deposits which were pressed or roded into the tunica media. No signs of nflammation were seen in the vessel wall fig. 5).

Discussion

According to the literature the common origin of acquired aneurysm of the coronary arteries is the atherosclerosis with plaque formation in the tunica intima possibly complicated by dissecting bleeding down into the tunica media. Other possibilities as origin of the acquired aneurysm are acute endarteritis in consequence of a metastatic spread acute periarteritis, periarteritis nodosa, and syphilitic or rheumatic arteritis.

In case 1 syphilis and rheumatic fever may well be excluded as aetiological possibilities partly due to the history and partly to the microscopical and macroscopical findings. Periarteritis also appears to be out of the question as no signs of myocardial or epicardial foci were demonstrable. The existing panarteritis must thus either have developed from an endarteritis or be periarteritis nodosa. The majority of the metastatic aneurysms of the coronary arteries have developed secondarily to acute or subacute endocarditis (1 3 4 10 23 25) and in an aneurysm wall of this kind numerous bacteria could be expected together with considerable destruction of the endothelial lining and formation of a mural thrombus. The present picture does not quite correspond to that of an endarteritis, first of all because there were no signs of endocarditis, and furthermore, because no bacteria could be demonstrated in the wall. The literature reports, however a few cases of arteritis in the coronary arteries without simultaneous endocarditis (7 17) with bacterial spread from distant foci. In the present case the clinical picture could very well resemble a septic focus in spite of several negative blood cultures. The possibility of a distant focus as the cause of a metastatic endarteritis should not be dismissed and according to the autopsy the sepsis might possibly have originated from the gallbladder and/or the bile ducts.

Many factors support the assumption that this was a case of periarteritis nodosa. It is well known that the changes are due to a panarteritis with infiltration of neutrophil and eosinophil leukocytes, fibrinoid degeneration, and marked tendency to necrosis. Since 1866 when Kussmaul and Meyer (11) described the classical picture of periarteritis nodosa in the coronary arteries in a 27-year-old male, several such cases have been reported, partly isolated in the coronary arteries (5 6 15 20 21) and partly associated with similar changes in other vessels (9 19 24)

All things considered 10 cases have been reported of aneurysms of the coronary arteries developed from periarteritis nodosa. Seven of these cases are reported within the last 2 decades, and five of the cases are practically identical with the present case (5 6, 1 20 21) Three of the cases were bilateral, and all the patients were children who either died of rupture of the aneurysm or acute myocardial infarction due to the development of a thrombus in the aneurysm.

In case 2 the development of the aneurysm was no doubt due to atherosclerosis. Macroscopical examination revealed no arteritis. Syphilis and rheumatic fever may also be eliminated. In all probability the origin of the aneurysm was the highly extended atherosclerosis of the coronary artery The formation of a fibrous plaque has occurred here with calcareous deposits, and due *to the pressure in the lumen* the calcified intimal part is pressed into

the underlying tunica media, which is gradually destroyed. This case thus belongs to the common type of coronary aneurysms

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Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From the Clinical Laboratory Aurora Hospital, Helsingfors, Finland

The Effect of Dilution on the Protein binding of Sulfonamides

By

Nils-Erik Saris

The relation between the protein-binding of sulfonamides and their antibacterial activity has been studied by a number of authors (1 2 3 5 7) These studies have led to the general view that the antibacterial activity is affected mainly by the concentration of the free unbound fraction while the extensive binding to proteins is a major factor in delaying the excretion of the long-acting sulfonamides. Especially instructive were the experiments of Newbould and Kilpatrick (5) in which the bacteriostatic concentration in a protein-free solution was lower than in the presence of plasma, but equal to the concentration of unbound sulfonamide.

In a recent paper Madsen et al (4) reported that the antibacterial activity of serum from patients to whom sulfonamides had been administered was closely correlated with the total concentration and equal to that of sulfonamide in a protein-free solution. They concluded that protein-binding was of minor importance with regard to antibacterial activity However the experimental technique used by these authors involved the testing of the bacteriostatic effect of *in vitro* serum dilutions over the range 1/20—1/800, a protein-free diluent being used. A consideration of the effect of dilution on the equilibrium between free and protein-bound sulfonamide seems warranted.

Submitted for publication October 16, 1963.

Scholtan (6) has applied the theory of protein-binding to the binding of sulfonamides and obtained experimental data which may be used in this consideration. The large number of binding sites makes an evaluation of individual mass-action equilibrium constants difficult. In relatively low therapeutic concentrations fewer sites are involved, and a graphical approach becomes feasible Straight lines are obtained when log r is plotted against log c_f, where r denotes the specific binding capacity in moles of sulfonamide bound per mole of protein and c_f the concentration of free sulfonamide. In the case of serum, log may be used instead of log $\bar{r}$ where c denotes the concentration of bound sulfonamide. c_b and c_f may even be expressed as mg/100 ml but it should be borne in mind that $_b$ is an approximation for and should be read as milligram of sulfonamide bound by the proteins in 100 ml of serum rather than as milligram per volume.

The straight lines obtained with the above plot correspond to the equation

$$\log c_b = \log k^* + n \log c_f$$

Dilution affects the system in two ways 1 it lowers c_f, and 2. it lowers k

Let us first examine the effect on $\bar{r}$. By dilution is not directly changed since the ratio sulfonamide bound per protein is not changed. $_f$ is lowered and the equilibrium thus disturbed,

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From Medical Department A (Head K. Brochner Mortensen) and the Fibiger Laboratory (Head J Kieler) University Hospital, Copenhagen, Denmark

Cytotoxic Factor and Complement Fixing Antibody in Thyroid Disease

By

Poul Halberg

The assumption that chronic thyroiditis (Hashimoto s disease) is an auto-immune disease is based on the occurrence of organ-specific antibodies in sera from patients with this disease, as demonstrated by classical serological techniques. As in other so-called auto-immune diseases the question arises whether these antibodies have in fact any importance in the development or the progression of the disease, or whether they should be considered a concomitant phenomenon.

The study of this problem calls for a more physiological thyroid preparation than the extracts of the organ used in the classical serological tests. Using cell cultures obtained from surgical specimens of thyroid glands Pulvertaft et al (9 10) and Irvine 4 5 found that sera from patients with Hashimoto s disease have a cytotoxic effect on human thyroid cells. It has also been shown by them that this complement-requiring cytotoxic factor may also be present in sera from patients with toxic goitres and myxoedema. However only a few attempts have been made to study systematically the incidence of the cytotoxic factor in sera from patients with thyroid diseases (6 10) The need for a standardized technique has been realized and titrations of cell population by means of standard sera have been described (6) However only very strongly toxic sera were used in these experiments, and titration was never carried beyond a serum dilution of 1 10 000.

In the present investigation the variability of the susceptibility of thyroid cells from various cases of non-toxic and toxic goitres to the cytotoxic factor was confirmed. This led to the development of an improved standardized method for the demonstration of the cytotoxic factor In this method the sensitivity of the cell material used was analyzed by only moderately strong standard sera which were diluted until cytotoxicity could no longer be demonstrated.

Material and methods

One hundred and fifty-eight sera from patients with various thyroid diseases were studied and an attempt was made to identify the cytotoxic factor with one of the thyroid anti-

Submitted for publication October 21 1963.

Table I The effect of dilution on the protein-binding of sulfonamides (for explanations, see text)

Drug	m	k* dl/ (mg)	Serum (10 mg drug/ 100 ml)		After dilution (1/100)	
			Free drug (mg/ 100 ml)	Protein-binding ( )	Free drug (mg/ 100 ml)	Protein-binding (%)
Sulfadimethoxine	0.513	16.30	0.56	96	0.061	39
Sulfamethoxypyridazine	0.522	7.20	1.36	86	0.080	20
Sulfadiazine	1 064	0.71	5.55	45	0.10	0.6

which leads to the release of some of the bound sulfonamide until a new equilibrium has been reached In table I the constants m and k^* of three sulfonamides estimated by Scholtan (6) are tabulated together with c_f and the percentage of sulfonamide bound at equilibrium at a concentration of 10 mg sulfonamide/100 ml serum and after dilution 1/100 The values have been calculated by means of the equation above. It is seen that as a result of dilution the binding of sulfon amide drops from 96 to 39 % in the case of sulfadimethoxine from 86 to 20 in the case of sulfamethoxypyridazine and from 45 to 0 6 in the case of sulfadiazine.

Data on the effect of dilution on k^* are scarce Scholtan (6) found a displacement in the log c_b — log c_f plot towards c_f without a change in the slope (m) of the lines when the albumin concentration was lowered From Scholtan's plots an m value of 0 82 and k^* values of 0 49 and 0.205 are obtained for sulfadiazine in 4 and 1 % albumin solutions, respectively At a concentration of 2 5 mg drug/100 ml 30 % would be bound in a 4 albumin solution but only 15 % in an 1 solution The change in k^* with a change in the protein concentration is indeed inherent in the considerations used in the calculation of the data in the table The matter is complicated by secondary effects such as the increase in the overall binding constants on dilution which may be due to a lessening of protein inter actions The effect of greater dilutions on k^* cannot therefore, be calculated before more experimental data are obtained.

It is evident from the above considerations that dilution may cause a considerable dissociation of protein-bound sulfonamide even in the case of sulfonamides which are almost completely bound by serum Great caution should be exercised in the interpretation of data obtained by the dilution technique

Summary

The influence of dilution of serum on the protein-binding of sulfonamides is discussed and the magnitude of the shift towards free sulfonamide assessed on the basis of the available data by calculation of some model examples.

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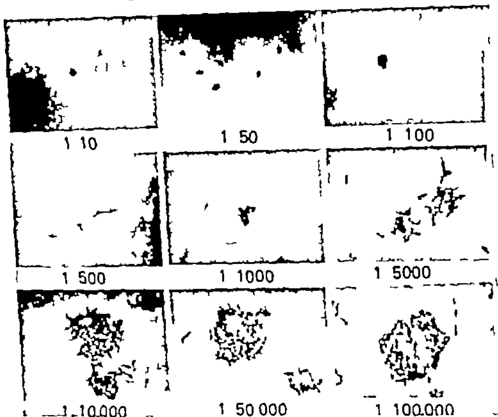


Fig. 3. Titration of cytotoxic serum, which has been added in concentrations of 1 10 to 1 100,000. I the cell cultures incubated with dilutions of the serum up to 1 500 no live, epithelioid cells are seen. In the cell cultures incubated with higher dilutions spread out, epitheloid cells are seen. Hematoxylin stain.

After straining through gauze the cells were centrifuged, the supernatant trypsin solution was removed and the cells resuspended in Parker' medium (TC 199 Difco) The cells were cultured directly on the walls of centrifuge tubes.

As source of complement fresh human serum to 10 % was added to each tube. Sera to be tested for cytotoxicity were likewise added to concentration of 10 The cultures were read after 18 hours. Live cultures revealed clumps of well spread out epitheloid cells dead cultures showed no cells t all or clusters of cells which had failed to spread out. I order to ensure the specificity of the cytotoxic effect all the cytotoxic sera were tested in number of negative control experiments. Cell populations which by titration with known cytotoxic sera were shown not to be susceptible to the cytotoxic factor were incubated with the sera. Only if these cells survived would the cytotoxicity of th sera be considered specific.

Complement fixation test (CFT) were performed as described by Roitt et al. (11) Passive hemagglutination tests (TRC) were performed using thyroglobulin-sensitized cells purchased from Burroughs Wellcome & Co.

Results

The cytostatic effect of the cytotoxic factor is illustrated in fig 1 a—d, which show a 12-hour old cell culture from a toxic thyroid before and 1 /₂, 3 and 6

Fig. 1. a) thyroid cell culture 12 hours after trypsinization. b), c) and d) same cells 1½, 3, and 6 hours after incubation with a serum from a patient with Hashimoto's disease and fresh normal serum.



Fig. 2. a) cell culture from the same thyroid as the cells shown in fig. 1. b) same cells 6 hours after incubation with fresh normal serum, but without serum from Hashimoto patient.

bodies demonstrated by classical serological technique.

Surgical specimens of toxic and non toxic (adenomatous and follicular) goitres were grown in vitro as described by Pulvertaft (8). The tissue was cut into minute pieces and subsequently trypsinized for 1½ hour at 37° C with constant magnetic stirring. Trypsinization was carried out with an 0.2 % solution of crystalline trypsin (250 Difco) dissolved in a salt solution without calcium and magnesium and buffered with bicarbonate at pH 7.5.

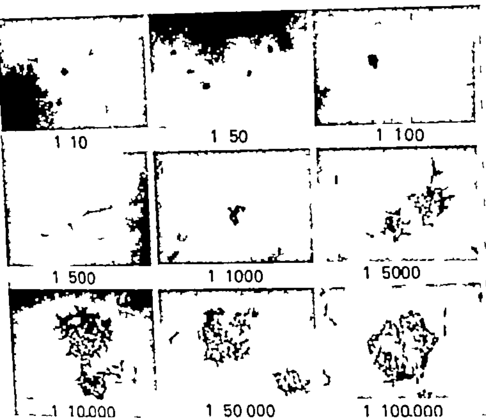


Fig 1. Titration of cytotoxic serum, which has been added in concentrations of 1 10 t 1 100,000. I the cell cultures incubated with dilutions of the serum up to 1 500 no live, epitheloid cells are seen. In the cell cultures incubated with higher dilutions spread out, epitheloid cells are seen. Hematoxylin stain.

After straining through gauze the cells were centrifuged, the supernatant trypsin solution was removed and the cells resuspended in Parker medium (TC 199 Difco). The cells were cultured directly on the walls of centrifuge tubes.

As source of complement fresh human serum to 10 % was added to each tube. Sera to be tested for cytotoxicity were likewise added to concentration of 10 %. The cultures were read after 18 hours. Live cultures revealed clumps of well spread out epitheloid cells dead cultures showed no cells t all or clusters of cells which had failed to spread out. I order to ensure the specificity of the cytotoxic effect all the cytotoxic sera were tested in number of negative control experiments. Cell populations which by titration with known cytotoxic sera were shown not to be susceptible to the cytotoxic factor were incubated with the sera. Only if these cells survived would the cytotoxicity of the sera be considered specific.

Complement fixation test (CFT) were performed as described by Roitt et al. (11). Passive hemagglutination tests (TRC) were performed using thyroglobulin-sensitized cells purchased from Burroughs Wellcome & Co.

Results

The cytostatic effect of the cytotoxic factor is illustrated in fig 1 a—d, which show a 12 hour old cell culture from a toxic thyroid before and 1 /₂ 3 and 6

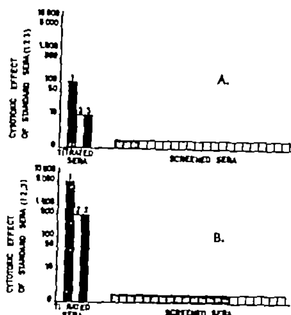


Fig 4 Titration of the cytotoxic effect of 3 standard sera (1 2 and 3) and screening of 22 other sera for cytotoxic effect using 2 cell populations (A and B) with different sensitivity to the cytotoxic factor

▭ No cytotoxic effect.
▭ Cytotoxic effect.

hours after the addition of a serum from a patient with Hashimoto's disease The action of the cytotoxic factor is seen The cells become coursely granulated and smaller with more distinct cell borders. Shortly after the last picture was taken the cells fell off the glass Fig 2 a and b show a control culture obtained from the same thyroid. To this culture only fresh normal human serum was added. Fig 2 a was taken at the same time as fig 1 a, and b was taken at the same time as fig 1 d. This culture was unaffected by the addition of normal serum alone

The sensitivity of each cell population i.e. cells derived from one thyroid gland was determined by titration cytotoxic standard sera being added in decreasing concentrations. Fig 3 shows the result of incubating a cell population with decreasing concentrations of a cytotoxic serum. The titre in this case was 500 the definition of a surviving culture being the presence of epitheloid cells. It soon turned out that there was a vast variation in the sensitivity of different cell populations to the same cytotoxic standard serum. In a number of experiments the titre could not be determined because the end-point of the titration was not clear The results of such experiments were discarded

Fig 4 shows how different the results can be if cell populations of varying sensitivity are used in testing the same sera for cytotoxicity On the other hand the relative sensitivity of the cell populations to different toxic sera is very constant. Consequently in order to compare the occurrence of the cytotoxic effect with the occurrence of thyroid antibodies demonstrated by serological methods the determination of the cytotoxic factor had to be standardized.

A standard sensitivity was chosen and defined as a cell population which when titrated for sensitivity with a standard cytotoxic serum gave a certain titre. If the cells in an experiment turned out to have exactly standard sensitivity all the results on the sera screened for cytotoxicity could be read without reservation. If, however the sensitivity of a cell population turned out to be above standard (its titre using the standard serum being above standard) only the live cultures could be read since these cultures had evidently been incubated with sera without cytotoxic effect even on cells of high sensitivity to the cytotoxic factor The dead cultures could not be read since these cultures might still have been alive if the sensitivity of the cells had been less, namely the standard value. Consequently the sera which killed the cultures in this experiment had to be tested again in a new experiment If the sensitivity of a cell popu

Table I. Incidence of cytotoxic factor (CTF), complement fixing thyroid antibody (CFT) and thyroglobulin antibody (TRC) in thyrotoxicosis, Hashimoto's disease, myxoedema and atoxic adenomatous goitres

	No. of positive tests			No. of pat.
	TRC	CTF	CFT	
Toxic goitre	10	24	18	66
Hashimoto's disease	24	23	22	25
Myxoedema	12	8	8	23
Atoxic (adenomatous and follicular) goitre	3	3	1	42

lation was below standard the dead cultures could be read, since these cultures had been incubated with sera the cytotoxic effect of which was exerted even against cells of low sensitivity. However the live cultures could not be read because they might have been dead if the cells had been more sensitive, namely of standard sensitivity. In this case sera yielding negative results had to be screened for cytotoxicity again with a new cell population.

The sensitivity of the cells decreased by about 50 in 24 hours. Consequently it was not possible to determine the sensitivity of cell population before it was used for the demonstration of cytotoxicity of the sera to be tested.

A total of 158 sera from patients with Hashimoto's disease, myxoedema, atoxic and toxic goitres were tested for the occurrence of the cytotoxic factor using the above mentioned principles. Thirty-five cell populations all derived from toxic goitres were used. The cell populations were standardized by titration by means of the same standard serum in each case.

Table 1 shows the incidence of the cytotoxic factor, positive complement fixation tests, and positive hemagglutinations tests in various thyroid diseases. The diagnoses of atoxic, toxic goitres, and Hashimoto disease were well established, since these diagnoses had been verified by histological examination in each case. The heading atoxic goitre refers to adenomatous and follicular goitres. The diagnosis of myxoedema was made by means of BMR, PBI [131] determinations, and I[131] examinations. Eight of these patients had congenital myxoedema, and the rest of the patients in this group had adult spontaneous myxoedema. One of the sera from the patients with congenital myxoedema had a low titre of TRC another serum was cytotoxic, but when titrated it was only weakly positive.

All sera from the patients with Hashimoto disease contained antibodies according to at least one of the tests the great majority of the sera contained antibodies according to all 3 tests. Very few of the sera from the group with atoxic (adenomatous and follicula) goitres contained antibodies.

Fig 5 shows that the same relative distribution of positive reactions was found in all 4 groups. All sera with a positive CFT were also cytotoxic whereas some of the cytotoxic sera had a negative CFT

On the other hand the occurrence of a positive TRC was unrelated to the occurrence of the cytotoxic factor or a positive CFT. This suggests that the cytotoxic factor and the complement-fixing antibody are identical, but the method of demonstrating the cytotoxic factor is

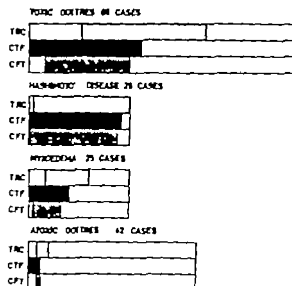


Fig 5 Sera from patients with toxic goitres, Hashimoto's disease myxoedema, and atoxic, adenomatous and follicular goitres tested for the occurrence of thyroglobulin antibody (TRC) cytotoxic factor (CTF) and complement fixing antibody (CFT)

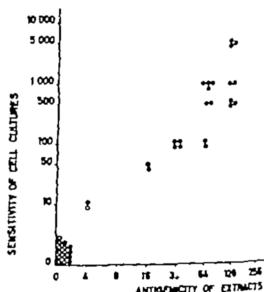


Fig 6. 42 atoxic (adenomatous and follicular) and toxic goitres tested for their sensitivity to the cytotoxic factor and for their complement-fixing antigenicity using the same standard serum in both reactions.
● Toxic goitres. ○ Atoxic goitres.

more sensitive than the method of demonstrating the complement fixing antibody On the other hand the thyroglobulin antibody demonstrated by the hemagglutination technique seems to be unrelated to the cytotoxic factor

Fig 6 shows the result of 42 toxic and atoxic goitres tested for their sensitivity to the cytotoxic factor as well as for their complement fixing antigenicity Each specimen was cut into minute pieces minced thoroughly and divided into two parts From one part a cell suspension was prepared after trypsinization and the cells were cultured after incubation with a standard cytotoxic serum in decreasing concentrations the sensitivity of the cells to the cytotoxic factor being determined after 18 hours. The other part of the tissue was extracted and its complement fixing antigenicity was determined by titration by means of the same standard serum as had been used for the titration of the sensitivity of the cells to the cytotoxic factor

All the 42 cases were well defined both clinically and histologically All the atoxic goitres were adenomatous or follicular none of them revealed signs of thyroiditis. Several attempts were made to trypsinize and culture thyroids with Hashimoto's disease but in all cases the cultures obtained were quite unsatisfactory and the sensitivity to cytotoxic sera could not be read with any degree of certainty

Sensitivity to the cytotoxic factor as well as complement fixing antigenicity was found in 23 cases of toxic goitre, whereas 4 in this group were neither sensitive nor antigenic. Of the atoxic goitres 11 were neither antigenic nor sensitive to the cytotoxic factor while 4 were active in both respects.

An attempt was made to plot the sensitivity of the 42 goitres to the cytotoxic factor against their complement-fixing antigenicity The result suggests a connection but statistically the significance of the correlation is low ($r = 0.55$)

The complement-fixing antigen is localized in the microsomal fraction. If the cytotoxic factor is an antibody and if it is identical to the complement-fixing antibody the corresponding antigen would have to be found in the microsomal fraction. Consequently it should be possible to absorb both the cytotoxic and the complement-fixing activity of a Hashimoto serum by means of a microsomal fraction obtained from a thyroid which was both antigenic and sensitive.

Table II shows 6 such experiments. Six different sera were absorbed by means of 6 different microsomal fractions, which had been obtained by centrifuging extracts at 150,000 × g for 2 hours after nuclei and mitochondria had been previously removed by centrifuging at 5 000 × g for 15 minutes. Many more experiments were made, but had to be discarded either because the cytotoxic sera became contaminated after absorption or because the sera became anticomplementary after absorption. In 5 cases both antigenicity and cytotoxicity were removed entirely by absorption. In serum No. 6 both qualities were present after absorption, but both titres had decreased.

The intracellular localization of the antigen responsible for the cytotoxic reaction raises the question whether damage to the cell membrane due to trypsinization is required for the establishment of contact between the antigen and the antibody. In order to elucidate this question thyroid tissue was explanted without trypsinization. I such cultures both epithelioid cells and fibroblast-like cells readily grew out, but since transitional forms could also be discerned it was considered impossible to characterize the cells. However t was quite clear that no differences could be seen between cultures exposed to known cytotoxic sera for up to 10 days

Table II. Cytotoxic effect and complement-fixing antibody of sera before and after absorption by means of microsomal fractions prepared from thyroids with known sensitivity to the cytotoxic factor and known antigenicity in the complement fixing reaction

Serum no.	Before absorption		After absorption	
	CFT	CTF	CFT	CTF
1	32	100	0	0
2	32	100	0	0
3	32	100	0	0
4	64	500	0	0
5	32	500	0	0
6	64	1,000	32	100

and cultures which had received normal sera only.

The susceptibility of the thyroid cells to the cytotoxic factor decreases soon after trypsinization. Already after 24 hours the titre has gone down to half of the original titre, and after a further 48 hours the cells are completely insensitive to the cytotoxic sera. This finding may indicate that a repair of the cell membrane has taken place, thus making the cells insensitive to the cytotoxic factor but further experiments rather suggested that the change in sensitivity is due to a loss of antigens during the 24—48 hour period following trypsinization. Thus, it was found that sensitivity to the cytotoxic factor could not be restored by retrypsinization after 3—5 days.

Discussion

Irvine (6) found that sera from 37 out of 39 patients with Hashimoto's disease, 20 out of 21 cases of spontaneous and post operative myxoedema two-thirds of 61 cases of thyrotoxicosis and 3 out of 27 cases of simple goitre were cytotoxic. Pulvertaft (10) found cytotoxic ctivity in 47 out of 50 sera from patients with Hashimoto's

disease in more than two-thirds of 35 cases of toxic goitres and in 6 out of 38 cases of colloid goitres In some respects the present results differ from those found by the above workers Only 8 out of 25 cases of myxoedema had the cytotoxic factor in the serum Eight of these had congenital myxoedema out of which the serum of only one was cytotoxic. If these cases were omitted still only 7 sera out of 17 in this group were cytotoxic, and only one-third of the sera from patients with thyrotoxicosis were cytotoxic. This difference is probably due to the standardization of the results in the present work. In the papers mentioned no allowance was made for the variation in the sensitivity of cells derived from different thyroids

Irvine (6) found that all of 16 toxic goitres and 3 out of 8 simple goitres were sensitive to the cytotoxic factor In the present work it was found that toxic goitres were not invariably sensitive, since 4 out of 23 toxic goitres revealed no susceptibility to the cytotoxic factor However the standard serum used throughout the experiments had only a moderately high titre of cytotoxicity In two of these experiments serum stronger than the standard serum was also used and it turned out to be cytotoxic in both cases. Four out of 15 atoxic goitres were sensitive to the cytotoxic factor The microscopic pictures of these goitres (at any rate the parts of them that were histologically examined) were not different from those found in the goitres which were not sensitive.

It has previously been suggested (2 6) that CFT and CTF are identical or closely related and that CTF is unrelated to the thyroglobulin antibody This assumption was based on the relative distribution of these antibodies and the finding that a microsomal fraction which was shown to be antigenically potent in the CFT could absorb the cytotoxic factor from a serum. The present findings are in accordance with these results. Out of 42 toxic and atoxic goitres tested for sensitivity to the cytotoxic factor and complement fixing antigenicity 27 were active in both respects, 15 were inactive in both reactions and no goitres were active in one reaction without being also active in the other one. Moreover a suggested but not statistically significant correlation was found between the titres of the two reactions. A number of absorption experiments suggested that both complement fixing antibody and cytotoxic factor could be removed by means of microsomal fractions prepared from goitres antigenically active in both respects. In one case absorption failed to remove all the complement fixing antigenicity and some degree of cytotoxicity was also left after absorption Finally the relative distribution of CFT CTF and TRC in 158 sera suggested a connection between CTF and CFT but no correlation between TRC and CTF

It seems reasonable to assume that the cytotoxic factor is an antibody and probably identical with the complement fixing antibody The fact that the complement fixing antibody is able to kill thyroid cells under certain conditions suggests that it might be of some pathogenic importance in the development of thyroiditis and not merely a concomitant phenomenon. However the demonstration of a cytotoxic effect on thyroid cells *in vitro* represents a model of the disease which is unsatisfactory in many respects. Thus, the sensitivity of the cells to the cytotoxic factor decreased rapidly and after 24 hours no cytotoxic effect could be demonstrated. This may be due to antigenic deletion, which frequently occurs in primary explants If this is the case, the need for

trypsinization might be explained by an increased permeability of the cell membrane allowing the cells to take up sufficient amounts of antibody before the intracellular antigen is lost. A similar need for rapid uptake of the antibody would not exist in vivo where the risk of antigen deletion is much smaller. Under these conditions a slow accumulation of the antibody may eventually lead to a cytotoxic immunological reaction.

The finding of the cytotoxic factor in sera from patients with toxic and simple goitres is a further argument against the cytotoxic factor being pathogenically active. The microscopic picture of these glands often reveals foci of thyroiditis, but apparently these lesions are rarely progressive because patients with simple goitres and toxic goitres rarely develop myxoedema. The cytotoxic factor found in sera from these patients could hardly be identical with the factor producing the progressive destruction of the thyroid seen in Hashimoto's disease. However the difference between the natural course of Hashimoto's disease and the focal lesions of thyroiditis may be quantitative rather than qualitative. Thus patients with Hashimoto disease may stay euthyroid for many years, and a number of patients with thyrotoxicosis do develop myxoedema. It has been suggested (3, 7) that a connection exists between the presence of thyroid antibodies in thyrotoxicosis and the subsequent development of myxoedema.

Summary

The possible pathogenetic importance of circulating thyroid antibodies in the development of thyroid disease was studied by means of cell cultures obtained by trypsinization of thyroid glands. These cell cultures were exposed to sera in which thyroid antibodies were found by classical serological techniques.

A specific cytotoxic factor could be demonstrated. It was found in almost all sera from patients with Hashimoto's disease and in / of patients with myxoedema and thyrotoxicosis, whereas sera from patients with adenomatous and follicular non-toxic goitres were cytotoxic in few cases. Comparison between the cytotoxic factor (CTF) and thyroid antibodies demonstrated by means of complement fixing reaction (CFT) and passive hemagglutination technique (TRC) showed a connection between CTF and CFT whereas the distribution of positive TRC's seemed unrelated to the two other reactions. Of 42 toxic and atoxic goitres tested for sensitivity to the cytotoxic factor and for complement fixing antigenicity most of the toxic specimens revealed both properties, whereas most of the atoxic goitres revealed neither. All the goitres had either both properties or neither. Plotting of sensitivity and antigenicity against each other suggested a connection but it was not statistically significant. Microsomal fractions prepared from thyroids both sensitive to the cytotoxic factor and antigenic in the complement fixation reaction could abolish or diminish the titres of cytotoxicity and complement fixing activity in sera with both of these properties. No cytotoxic effect could be demonstrated in trypsinized cell cultures older than 48 hours, even after retrypsinization and no cytotoxic effect could be demonstrated if explants of thyroid tissue were used instead of trypsinized cells.

Acknowledgement

Aided by grant from the Danish Foundation for the Advancement of Medical Science and grant from F. L. Smidth & Co. A/S' Jubilæumsfond.

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Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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The Relationship Between the Cytology and the Immuno-electrophoretic Pattern in 105 Cases of Myelomatosis

By

Aa. Drivsholm and J. Clausen

The connection between serum immuno-globulins and plasma cells has been known for a long time. Already in 1913 Huebschmann (26) on th basis of investigations of the spleen advanced the theory that plasma cells are connected with immunologic reactions. In 1937 Bing and Plum (6) demonstrated increased numbers of plasma and reticulum cells in bone marrows from patients with hyperglobulinemia varying in etiology Bing and Plum (6) also put forward the hypothesis that serum globulins are formed by plasma cells. Four years later Bjorneboe and Gormsen (7) demonstrated that experimentally produced hyperglobulinemia in rabbits was associated with a distinct proliferation of plasma cells. These findings were later confirmed and extended by Fagraeus (15)

I 1947 Martin (37) reported that proteins extracted from a plasmocytoma contained fraction which by ultra centrifugation was identical with the abnormal serum protein component found in the patient's serum Later Vazques (57) by means of immuno-fluorescence studies confirmed the presence of γ-globulins in myeloma cells. However neither of these studies allowed it to be decided whether the γ-globulins were formed by or merely deposited in the myeloma cells. That myeloma protein is actually *formed* by plasma cells was proven by Meyer (40) in 1957 After plasma cells from a myeloma patient had been incubated with C^{14}-labeled lysine a protein could be isolated from the surrounding medium which had the properties of a Bence-Jones protein and contained 95 % of the activity This protein fraction was identical with the Bence-Jones protein present in the patient urine. These experiments confirmed the theory of Magnus-Levy (35) that myeloma cells are the production sites for Bence Jones proteins.

Following these investigations the next step was to tudy the possible relationship

Submitted for publication October 21, 1963.

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The term "plasmocytic reticulum cells" is used for reticulum cells with a plasmocytoid appearance, i. e. with a more basophilic cytoplasm and a more condensed chromatin than normally seen in reticulum cells. The identification of plasmocytic reticulum cells from ordinary reticulum cells is difficult and a large measure a matter of subjective evaluation. The same applies to the differentiation between immature plasma cells and plasmocytic reticulum cells.

The term myeloma cell as used in this paper includes both plasma cells and plasmocytic reticulum cells.

Neutrophilic segmented granulocytes were counted in order to obtain a rough estimate of the admixture of blood. Unclassifiable cells were counted in order to check the validity of the myeloma cell counts (see later). All the bone marrow cells not listed were counted together in the group remaining cells. This group also included naked nuclei, even if they had the features of plasma-cell nuclei, because exact morphologic characterization of naked nuclei is impossible.

Material from two bone marrow aspirates was studied in 20 patients.

In order to decide whether the characteristic morphological features, listed under point 1 a, b, & c, can be seen in normal bone marrow plasma cells, bone marrow samples from ten normal individuals and from five patients with reactive plasmocytosis were studied. The ten normal smears are part of a larger series of normal individuals (18); the preparations with reactive plasmocytosis originate from 5 patients in whom no evidence of myelomatosis was found at autopsy.

Periodic-acid-Schiff-staining (*P.A.S.*) was performed on smears previously stained with May-Grunwald-Giemsa (cf. Hayhoe et al. 22)). The intranuclear inclusion bodies were photographed before and after P.A. S.-staining. The P.A.S.-staining procedure was performed as follows (39):

1) Treatment with distilled water for 10 min.
2) Periodic-acid for 10 min.
3) Distilled water twice, for 3 min. each.
4) Schiff' reagent for 60 min.
5) Sulphite water three times, for 4 min. each.
6) Distilled water for 5 min.
7) Counterstaining for 10 min. with 0.25 % malachite green dissolved in water.
8) Distilled water twice for 3 min. each.
9) 96 % alcohol.
10) Xylene.

Periodic-acid 1.6 g periodic-acid + 40 ml distilled water + 140 ml absolute methanol + 20 ml 1/5 M sodium acetate.

Schiff' reagent. (can be kept at 4 C. for approx. 1 month) 1 g diamond fuchsin (Merck) + 200 ml boiling water. Shake the mixture for one minute. Cool. After filtration 1 ml thionyl chloride is added and the mixture is stored for 12 hours in the dark. Then 2 g of charcoal is added and the mixture is shaken for one minute and filtered. The solution obtained should be waterclear.

Sulphite water (prepared immediately prior to use) 2 g of potassium metabisulphite are dissolved in 20 ml distilled water and diluted with 500 ml distilled water and 5 ml concentrated HCl.

Prior to the P.A.S.-staining the samples were treated with amylase to exclude P.A.S. staining of glycogen.

Biochemical method

Immuno-electrophoresis was performed by the method of Grabar and Williams (19) as modified by Scheidegger (49) (see also the reviews by Clausen (11, 12)).

The antisera used were the following:

1) A horse antiserum against normal pooled human serum from the Pasteur Institute (No. 15.483).

2) A goat antiserum against normal pooled human serum, produced by subcutaneous injection of 0.2 ml pooled human serum from 10 normal individuals every two weeks for three months. The first injection was followed by subcutaneous injection of 0.5 ml complete Freund's adjuvant (DIFCO). The antiserum was obtained by puncture of cubital vein exactly 8 days after the last injection.

3) A rabbit anti γ_{ss}-globulin produced as described above. The γ-globulin used was isolated by preparative electrophoresis in Pevicon C. 870 as described by Muller-Eberhardt (41). After running time of 18 hours, the immunologically pure γ-globulin could be extracted with saline from a 3 cm wide band situated most cathodically in the protein pattern. The γ-globulin was brought to 5 % w/v by vacuum dialysis.

between morphology of the myeloma cells and the types of myeloma serum proteins (3 10 13 17 21 21 a, 27 31 33 34 36 42 45, 50 51 56 59 61 61 a, 62) In all these studies, myeloma proteins were classified according to their electrophoretic mobility and myeloma cells according to size, maturity and nucleo-cytoplasmic ratio The results are highly contradictory In some reports (10 33 36 42 51 62) a correlation was found between protein types and cytologic characteristics but results differed from one study to another In the remaining studies (3 13 17 21 21 a 27 31 34 45 50 56 59 61 61 a) no correlation was found

With the introduction of immuno-electrophoresis it became possible to obtain a better classification of the protein patterns in myelomatosis This classification (vide infra) enabled Paraskevas et al (44) to demonstrate a specific cytologic feature in patients with myelomatosis of the γ_{1-A} type, characterized by one or more of the following features 1) plasma cells with intranuclear inclusions 2) flame cells (flaming plasma cells) and 3) thesaurocytes (cf fig 3 d)

The purpose of this report is to present data on the relationship between the immuno-electrophoretic pattern and the occurrence of morphologic changes in myeloma cells visible in ordinary May-Grünwald-Giemsa-stained bone marrow smears.

Material and methods

Bone marrow smears from 105 patients with myelomatosis were studied. In all cases the diagnosis was established by the demonstration of myeloma cells in the marrow smears. In doubtful cases the diagnosis of myelomatosis was confirmed by biopsy from a radiologically demonstrated osseous lesion or by autopsy Two-thirds of the patients were admitted to the Medical Department A, Rigshospitalet, between 1949 and 1963, the remaining patients were admitted to other hospitals.

The cytological studies were done without any knowledge of the immuno-electrophoretic results. Ordinary May-Grünwald-Giemsa-stained bone marrow smears, mainly from the first diagnostic puncture, were used. The cytologic investigations were performed at a magnification of 750 times according to following scheme.

1) A detailed survey of the whole slide with particular attention to a) Inclusion bodies in plasma cells. b) Flame cells. c) Thesaurocytes.

2) Differential count of 1 000 nucleated bone marrow cells which were categorized as follows

a) Plasma cells the number of bi- and multinucleated cells was recorded separately b) Plasmocytic reticulum cells. c) Neutrophilic segmented granulocytes. d) Unclassifiable cells. e) The remaining cells.

3) Attempt to classify the myeloma cells with respect to differentation and maturation.

Intranuclear inclusion bodies in plasma cells occur in two different forms which will be referred to as type 1 and 2

Type 1 are solitary or multiple bluish-grey hyaline inclusion bodies, surrounded by a rim of dark material (fig 3 e & f) *Type 2* are round, light brown, solitary inclusion bodies without a rim (fig 3 g & h)

It must be emphasized that great care is necessary in order not to overlook plasma cells with intranuclear inclusion bodies, because their frequency usually is less than one per cent of all plasma cells.

Flame cells are plasma cells in which part of or all the cytoplasm is acidophilic. These changes may give rise to a "flaming" appearance of the cytoplasm (fig 3 a, b & c) These cells should not be mistaken for the plasma cells with disruption of the cytoplasmic membrane and a slight eosinophilia in the margin which can be seen in the periphery of most bone marrow smears.

Thesaurocytes are large plasma cells with small and pycnotic nucleus and a homogeneous, greyish cytoplasm, which is divided into segments by thin basophilic trabeculae (Paraskevas et al. (44)) A typical thesaurocyte is shown in fig 3 d.

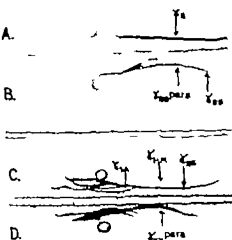


Fig. 1 Immuno-electrophoresis of serum from patient (M. J.) with γ1M-paraproteinemia. A Immuno-electrophoresis of normal human serum developed with an antiserum against human γ1M-globulin. B Immuno-electrophoresis of the patient serum, developed as A. The deflecting γ1M-globulin precipitation bow can be seen. C As A developed with goat antiserum against normal pooled human serum. D As B, developed as C. The deflecting γ1M-globulin-precipitation bow can be seen, associated with decrease in the γ-globulins with slow mobility and in the γ1A (beta2A) globulin and γ1M (beta2M). The precipitation bow for the last-mentioned proteins are further away from the antibody reservoir than usual and are shortened in the anode-cathode directions.

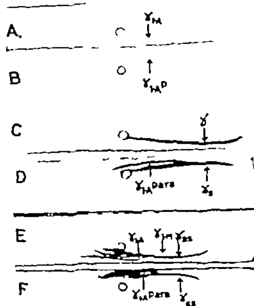


Fig. 2 Immuno-electrophoresis in case of γ1A (beta2A) paraproteinemia (patient C. C. H. A Immuno-electrophoresis of normal human serum developed with strictly specific antiserum against γ1-A. B Immuno-electrophoresis of serum from the patient, developed as A. The slightly deflecting γ1-A precipitation bow can be seen. The precipitation lines correspond to those found in D and F. C Immuno-electrophoresis of normal human serum, developed with an antiserum against γ-globulin. D Immuno-electrophoresis of serum from the patient developed as C. In the intermediate zone between the slow area and the fast area an accentuated deflecting precipitation bow can be seen. The abnormal bow must have immunologic groups in common with normal gamma globulin. These groups are those in common between γss and γ1A because the paraprotein can also be developed with strictly specific antiserum against the γ1-A globulin as demonstrated in B, E & F. The same as C and D now developed with goat antiserum against normal pooled human serum. The paraprotein precipitation bow can be seen.

ulinemia Waldenström but rarely encountered in patient with myelomatosis.

In 21 patients the γ-type of paraprotein (Bence Jones protein) was demonstrated. In 8 cases it was the only finding. In 10 cases this micromolecular paraprotein was found together with γss-paraprotein and in two cases with a γ -paraprotein. One patient had two -paraprotein components.

Cytologic investigation

The results of these studies can be seen from table II. Intranuclear inclusion bodies were found in 31 of the 103 cases. In 9 cases both bluish-grey inclusion bodies surrounded by a rim of dark material (referred to as type 1) and light-brown inclusion bodies without a rim (referred

Table I. Distribution of 105 cases of myelomatosis according to type of paraprotein in the serum. The ten patients with γ_{SS}- and γ_{μ}-paraprotein (10⁺) and the two patients with γ_{1-A} and γ_{μ} (2⁺⁺) appear twice. For further details see the text

Type of para protein	Associated components					
	None	γ_{SS}	γ_{1-A}	γ_{1-M}	γ_{μ}	Total
γ_{SS}	57	2	0	0	10	69
γ_{1-A}	24	0	0	0	2	26
γ_{1-M}	1	0	0	0	0	1
γ_{μ}	8	10	2⁺⁺	0	1	21

4) A rabbit anti γ_{1-A} (β_2 A) globulin antiserum from Behringwerke, Marburg/Lahn, Germany. This antiserum was made strictly specific against the γ_{1-A}-globulin by absorption in the proportion 10:1 with immunologically pure γ-globulin. In this way antibodies common to the γ globulin and the γ_{1-A}-globulin were absorbed.

These antisera were employed to reveal paraproteinemia. A paraprotein (M-component (24)) is an immuno-globulin lacking some immunologic groups, present in the corresponding normal immuno-globulin (47 a).

All sera subjected to immuno-electrophoresis were studied undiluted as well as diluted 4:1 with saline. In both cases immuno-electrophoresis was run in duplicate with the horse antiserum as well as with the goat antiserum. In this way all paraproteins with slow γ-mobility could be diagnosed with certainty. All paraproteins with intermediate γ-mobility as well as those with β-mobility were further checked immuno-electrophoretically with the γ_{SS} and the γ_{1-A} antiserum to differentiate paraproteins of the γ_{SS}-type from those of the γ_{1-A} type.

In a few cases these methods did not give any conclusive information because of complex formation (aggregation) of the paraprotein with another protein, e.g. with albumin or γ-globulin. In these cases the serum was treated in the proportion 10:1 with a neutralized 10 % cysteine hydrochloride solution, freshly prepared. After this treatment the scheme described above could reveal the real character of the paraprotein.

The criteria used for the immuno-electrophoretic identification of paraproteinemia (the presence of an M-component in serum) were the well established typical changes in the shape and position of the precipitation bow of one of two immunoglobulins: the γ_{SS} or the γ_1 A-globulin. Thus a paraprotein can be detected in the immuno-electrophoresis by the presence of an abnormal precipitate, located in a small, well-defined interval of mobility near the through but which anodically and/or cathodically fuses with the corresponding normal immuno-globulin precipitate. This abnormal precipitate appears when an excess of a defect immuno-globulin antigen diffuses against the antibody in the gel. Because there is an excess of antigen and because the antigen-antibody complex is soluble, the maximal precipitation is displaced toward the antibody reservoir. The γ_{μ}-paraprotein (Bence Jones protein in the serum) could be demonstrated immuno-electrophoretically as a very faint precipitation line located extremely near the antibody reservoir in any interval of mobility. This reflects the low molecular weight of the protein and its high diffusion rate in agar gel. This precipitation line fuses completely with the normal γ-globulin precipitation bow.

Results

Immuno-electrophoresis

Paraproteins were demonstrated in all 105 cases. Sera from 15 patients contained two paraproteins. The results are shown in table I. Sixty nine patients had a γ_{SS}-paraprotein. Fifty-seven of these had one paraprotein, 10 patients had both a γ_{SS} and a γ_{μ} paraprotein (Bence-Jones protein) and two had two γ_{SS} paraprotein components. Fig. 1 illustrates the immuno-electrophoretic findings in a patient with a γ_{SS}-paraprotein.

Paraproteinemia of the γ_{1-A} type was found in 26 patients, in 24 patients as the only abnormality. Fig. 2 shows the immuno-electrophoretic findings in a patient with γ_{1-A} paraprotein. One patient had a paraprotein of γ_{1-M}-type, a paraprotein which is typical for macroglob-

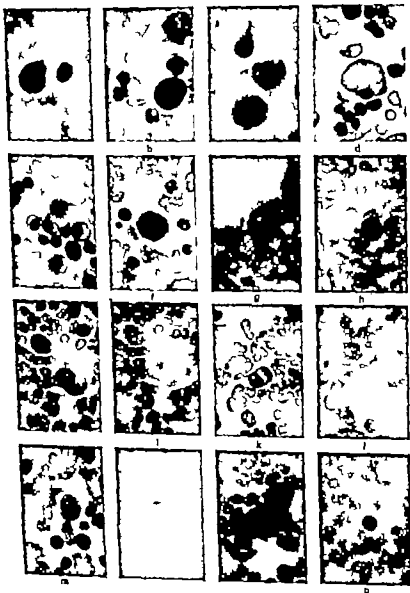


Fig. 5. Legend, see p. 614

Table II Distribution of 105 cases of myelomatosis according to cytologic findings Type 1 are bluish-grey intranuclear bodies (i i b) surrounded by a rim of dark material type 2 are light brown i i b without a rim

Plasma cells with i i. b	
Type 1	18
Type 2	4
Type 1+2	9
	31
Flame cells	22
Thesaurocytes	29
No special findings	74

Table III Correlation between cytology and types of paraprotein in 31 patients with intranuclear inclusion bodies. For further details see the text

Type	Cytological findings in plasma cells					
	Intranuclear inclusion bodies				Flame cells	Thesaurocytes
	1+2	1	2	Total		
γ_1–A	9	14	3	26	18	25
γ_{ss}	0	3	1	4	3	3
γ_μ	0	1	0	1	1	1
Total	9	18	4	31	22	29

Table IV Distribution of patients according to percentage of myeloma cells in bone-marrow smears. Myeloma cells comprise plasma cells and plasmocytic reticulum cells

Myeloma cells %	No. of cases
< 4.9	1
5 — 9.9	15
10 — 19.9	19
20 — 29.9	24
30 — 39 9	23
40 — 49.9	10
50 — 59.9	6
60 — 69.9	3
70 — 79.9	2
80 — 89.9	0
90 — 99.9	2
Total	105

to as type 2) were present. In 18 cases all the intranuclear inclusion bodies were of type 1 in 4 cases of type 2 Flame cells were found in 22, and thesaurocytes in 29 cases. All patients with flame cells and thesaurocytes also had intranuclear inclusion bodies.

Fig 3 a) I.M P Typical flame cell and plasma cell with ordinary cytoplasmic basophilia. b) I.M.P Flame cell with an eccentric pycnotic nucleus. Thesaurocyte-ill appearance of the flame cell and of the big myeloma cell at the top. c) C.R.C. Two flame cells. The cytoplasm in the periphery is homogeneous and stains pink. The central part is vacuolated and stains blue as usual. Note the structureless naked nucleus at the top. d) M.E.P Typical thesaurocyte with eccentric pycnotic nucleus, and large homogeneous cytoplasm divided into compartments' by thin basophilic trabeculae. e) K.J Plasma cell with two intranuclear inclusion bodies. Note the nucleolus between the intranuclear inclusion bodies, which are bluish-grey and surrounded by a rim of dark material (referred to as type 1) f) B.J Big myeloma cell with four nuclei, two of which contain an intranuclear inclusion body (type 1) g) J J P Myeloma cell with a light-brown inclusion body without a rim (referred to as type 2) h) J J P Myeloma cell with very big intranuclear inclusion body (type 2) i) G C. M. Big myeloma cell with inclusion body (type 1) j) G C.M. Fig after P.A.S staining. Note the P.A.S. positive globules surrounded by a P.A.S.-negative rim corresponding to the dark rim in fig i. k) T.L. Plasma cell with an intranuclear inclusion body (type 1) in patient with γ_{ss}-myelomatosis. l) T.L. Fig k after P.A.S. staining Note that the globule is P.A.S -negative m) J.A.E. Morular cell. The pycnotic nucleus is compressed and pushed towards the periphery of the cell. Honeycomb appearance of the greyish cytoplasm. n) J.A.E. Morular cell after P.A.S. staining Same patient as in fig m, but not the same cell. The cytoplasm, greyish in the M G G -staining, is P.A.S positive. o) M.E.P Crimson homogeneous mass of cytoplasm between the two myeloma cells, one of which has two nuclei. p) C.G.H. Structureless naked nucleus with an intranuclear inclusion body (type 1) For further details of fig o and p. see the text. j & l) P.A.S. stained smears, all others May-Grunwald-Giemsa-stained. k & l) From a patient with γ_{ss}-myelomatosis. All other figures from patients with γ_1–A-myelomatosis. All photos enlarged 400 times).

Correlation between cytology and type of paraproteinaemia

As shown in table III 26 of 31 patients with intranuclear inclusion bodies had a paraprotein of the γ_{1-A}-type. Of the remaining 5 4 had a paraprotein of the γ_{ss}-type and one had a γ-paraprotein.

All patients with a paraprotein of the γ_{1-A}-type had intranuclear inclusion bodies. Neither type 1 nor type 2 were specific for the γ_{1-A}-paraprotein type.

Differential counting

The percentage of myeloma cells in the single bone marrow smears can be seen from table IV

In one case, only 3 8 myeloma cells were found in the diagnostic bone marrow specimen. In this case the diagnosis of myelomatosis was confirmed by autopsy

Unclassifiable cells never exceeded 2 %. Thus, even if all unclassifiable cells should have belonged to the plasma cell series, this would not materially influence the percentage of myeloma cells in the differential counts.

Attempts to classify the myeloma cells with respect to differentiation and maturation were largely unrewarding because of the marked cytologic variability within a single smear

In part these difficulties have been overcome by detailed analysis including cytometric studies of 50 myeloma cells in 53 of the 105 bone-marrow smears. These results will be published separately (14)

Inclusion bodies in the cytoplasm of plasma cells were seen in many cases, most often as round, bluish-grey inclusion bodies resembling the above mentioned intranuclear inclusion bodies type 1 but often without the black rim. These inclusion bodies were found most frequently in bone marrows from γ_{1-A} paraproteinemia but also in several cases of γ_{ss}- and γ_{s}-paraproteinemia.

Morular cells (63) and Mott cells (4) were noted in a few cases and eosinophilic Russel-bodies (50) in 2 cases.

In some smears heavy eosinophilic masses of cytoplasm without a nucleus were found, often in close contact with myeloma cells (fig 3 o) A few of these smears contained structureless naked nuclei (fig 3 p)

In 20 cases two bone-marrow smears were studied. The percentage of myeloma cells in different specimens from the same patient varied moderately If plasma cells with intranuclear inclusion bodies, flame cells or thesaurocytes were found in one aspirate these features were invariably present in the other aspirate.

Investigation on normal bone marrow specimens

Careful study of ten normal bone marrow preparations failed to reveal plasma cells with intranuclear inclusion bodies, flame cells and thesaurocytes, nor were such cells found in the bone marrows from five patients with reactive plasmocytosis.

Periodic-acid-Schiff-staining

P.A.S.-staining was performed on bone marrow specimens from eight patients with intranuclear inclusion bodies in the plasma cells and γ_{1-A}-paraproteinemia. In all 8 cases P.A.S.-positive inclusion bodies were found (see fig 3 j)

The P.A.S.-staining was also performed on bone marrow smears from 2 of the 5 patients who had intranuclear inclusion bodies and paraproteinemia of the type γ_{ss} and γ (one of each type) In both cases the intranuclear inclusion bodies were P.A.S.-negative (see fig. 3 l)

Bone-marrow specimens from 10 patients with myelomatosis of the γ_{ss}-type

The flame cell was recognized many years ago. Undritz (55) published the first color picture of flame cells and described them as "atypisch gefärbte Zellen, ein gelegentlicher Befund bei verschiedenen Krankheiten". As stressed by Paraskevas et al. (44) it cannot simply be a staining anomaly because these cells are found also in preparations where the majority of plasma cells and all other cell forms have normal staining properties. The pH used in the staining procedure may influence the intensity of these changes. In the present study M. G. G.-staining was performed at pH 6.5 whereas Paraskevas et al. stained at pH 7.0. This might explain why we found more flame cells than Paraskevas et al. Electron microscopy studies by Bessis et al. (5) indicate that flame cells have stored large amounts of secretion products in their ergastoplasm. According to Bessis et al. flame cells are not specific for γ -myelomatosis but occur most frequently in this form. This agrees with our results. Brittin et al. (8) observed neither thesaurocytes nor flame cells in 6 cases of myelomatosis, 3 of which had γ_{1}- paraproteinemia, whereas they found these cells in 2 cases with reactive plasmocytosis. These results differ from those of the present study. No details of the staining procedure are given by Brittin et al. nor are the diagnoses of the 2 cases with reactive plasmocytosis stated. Rask-Nielsen et al. (47) found flame cells in preparations from mice with paraproteinemia but not in cases with γ_{1A}-paraproteinemia. From the above-mentioned results and the results of the present study one may conclude that the presence of flame cells is closely connected with the occurrence of γ -paraproteinemia. As pointed out by Heremans (25) it is unlikely however that the γ_{1-A}-paraprotein itself causes the flamed appearance of the plasma cells. It seems more likely that the flamed cytoplasm can be explained from intracellular presence of amyloid. This could explain the occurrence of flame cells also in cases of myelomatosis associated with paraproteinemia other than the γ_{1-A} type and why it is not possible to demonstrate flame cells in all cases of γ_{1-A}-paraproteinemia. The hypothesis of the intracellular storage of amyloid is in agreement with the data of Zlotnick and Tal (64) who found flame cells in 28 of 48 mice with experimental amyloidosis, the cytoplasm of these cells staining metachromatically with methylene blue. Unfortunately the report of Zlotnick et al. (64) does not give any data on the serum-immuno-globulins.

The intranuclear inclusion bodies in the plasma cells were first described by Apitz (1) who believed the inclusion bodies — identical with our inclusion bodies type 1 — to be Russell bodies. He concluded that the intranuclear inclusion bodies were different from nucleoli and might be related to an abnormal protein metabolism of the nucleus. Vogt (58) in a case of myelomatosis with numerous Russell bodies found what appears to be intranuclear inclusion bodies but referred to them as "nucleoli". None of these authors give any data on the serum proteins. Klinghofer and von Boroviczény (28) described "an atypical plasmocytoma" with intranuclear inclusion bodies and without paraproteinemia. Maurer (38) found intranuclear inclusion bodies in normally differentiated bone marrow cells in a patient with an abnormal protein peak. No information was given about the type of paraprotein. Nor does Bessis et al. (5) give any information about this in 2 cases with intranuclear

were also P.A.S.-stained. No P.A.S. positive structures were found in the plasma cells of these cases.

Finally, P.A.S.-staining was applied to one smear with Mott cells and one smear with Morular cells. The vacuoles in the Mott cells were P.A.S. negative, while the greyish inclusion bodies in the Morular cells were P.A.S. positive (fig. 3 n).

Discussion

Previous attempts to correlate the protein changes with the morphology of the plasma cells in myelomatosis have yielded inconsistent results. This is not surprising since until a few years ago the classification of myeloma proteins was based mainly on their electrophoretic mobility, i.e. on strictly physico-chemical properties. As not two myeloma proteins are alike with respect to their molecular size and structure (see the reviews by Putnam (46) and Fahey (16)) classification of myeloma proteins appeared to be insufficient. It was therefore, attempted to characterize myeloma proteins by immunological methods. In 1956 Korngold and Lipari (29) reported that myeloma proteins can be divided into 3 main groups by means of diffusion in-gel methods. This classification was later shown to be of great significance when by means of immuno-electrophoresis it was demonstrated that also normal human serum contains different types of immuno-globulins (Grabar et al. (20), Heremans (23)). In this way it became possible to relate the abnormal changes described by Korngold (29, 30) to certain well-defined normal immunoglobulins. For clinical purposes immunoelectrophoresis has proved more suitable than immuno-diffusion for the demonstration of changes in serum proteins in myelomatosis. Therefore, in this report myeloma proteins were classified according to the results of the immuno-separation.

The results of the present study agree with earlier reports. Waldenström (60) in a series of 142 patients with 129 definite and 13 probable myelomas found 30 cases with γ_{1-A} paraproteinemia, 6 with γ_μ paraproteinemia (Bence-Jones proteinemia) and 4 cases of γ_{1-M}-paraproteinemia (2 of which were probable myelomas). Among 140 cases of myelomatosis Ossermann and Lawlor (43) found 30 % with γ_{1-A} paraproteinemia.

Our cytologic studies were primarily concerned with the occurrence of plasma cells with intranuclear inclusion bodies, flame cells and thesaurocytes. The term "thesaurocyte" was introduced by Paraskevas et al. (44). According to Paraskevas a thesaurocyte is a plasma cell which has stored large amounts of globulin in its cytoplasm. This cell corresponds exactly in appearance to the "large lobulated, striated storage type of myeloma plasma cell" described by Bayrd and Bennett (2) in a patient with myelomatosis and amyloidosis and is similar to a type of plasma cell which Bussi and Buttura (9) found in the bone marrow of a patient with Weber-Christian's disease. It will appear from fig. 3 d that in typical cases these cells are so characteristic that they cannot be mistaken for any other cell. However, in most cases these cells are less typical and all kinds of intermediate forms between thesaurocytes, flame cells and plasma cells with a trabecular cytoplasmic structure can be seen. In our opinion these cells are difficult to delineate sharply. This may be the reason why Paraskevas et al. (44) found thesaurocytes less frequently than we do.

Summary

Sera from 105 cases of myelomatosis diagnosed from bone marrow examination, were studied by immuno-electrophoresis. Paraproteins were demonstrated in all sera. γ_{ss}-paraprotein was found in 69 patients, γ_{1-A} in 26, and γ (Bence-Jones protein in the serum) in 21 patients. In 9 patients γ was the only demonstrable paraprotein. In one patient a γ_{1-M}-paraprotein was found. Fifteen patients had two paraprotein components.

Bone marrow from the same patients were studied with respect to the occurrence of intranuclear inclusion bodies in plasma cells, flame cells and thesaurocytes. Such cells were observed in 31 patients of which 26 had paraproteinemia of the γ_{1-A} type 4 of the γ_{ss}-type and 1 of the γ type. In contrast, these cells could not be found in bone marrows from 10 normal individuals and from 5 patients with reactive plasmocytosis.

It is concluded that in all patients with myelomatosis of the γ_{1-A} type plasma cells with intranuclear inclusion bodies are found. In addition many of these marrows contain flame cells and thesaurocytes. However none of these changes in the plasma cells are absolutely specific for the γ_{1-A}-group.

The intranuclear inclusion bodies in the γ -group are P.A.S. positive. Intranuclear inclusion bodies, which in rare cases were found in myelomatosis of the γ_{ss} and γ -types, were P.A.S. negative. The significance of the findings has been discussed.

Acknowledgements

The authors wish to thank H. Gormsen, M. D., The Institute of Forensic Medicine, University of Copenhagen, and J. Bichel, M. D., The Cancer Chemotherapy Division, Radium Center University of Aarhus, for making bone-marrow smears available to us.

Aided by grants from Anders Hasselbalchs Fond til Leukæmiens Bekæmpelse and Lady Tata Memorial Trust.

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inclusion bodies out of 13 cases with myelomatosis and paraproteinemia. Paraskevas et al. (44) described plasma cells with intranuclear inclusion bodies in several patients with paraproteinemia all of the γ_{1-A} type. As the inclusion bodies were P.A.S. positive, they concluded that the intranuclear inclusion bodies are connected with the synthesis of globulin especially rich in carbohydrates. This is in agreement with the fact that we found cells with intranuclear inclusion bodies in all our patients with γ_{1-A} paraproteinemia and that the intranuclear inclusion bodies in these cases are P.A.S. positive. This theory too agrees with the fact that P.A.S. positive intranuclear inclusion bodies have been observed by several authors, first by Lelback (32) in the lymphocytic reticulum cells of Waldenström's disease, which is characterized by the occurrence of γ_{1-M}-paraprotein rich in carbohydrates. However intranuclear inclusion bodies have also been reported in paraproteinemia other than the γ_{1-M}- and γ_{1-A} types. Brittin et al. (8) found intranuclear inclusion bodies in 12 patients with various forms of paraproteinemia (γ_{1-A}, γ_{1-M}, γ_{SS} and γ_{μ}) and in one case with leukemia and what appeared to be a reactive plasmocytosis. In the present series intranuclear inclusion bodies could not be detected in the 10 normal cases and the 5 cases with reactive plasmocytosis studied. The patient of Brittin et al. (8) with leukemia and reactive plasmocytosis resembles one of our patients with intranuclear inclusion bodies and γ_{1-A} paraproteinemia. Initially this patient had a chronic lymphatic leukemia but subsequently developed myelomatosis (both diseases were demonstrated at autopsy). We too find intranuclear inclusion bodies in patients with myelomatosis and γ_{SS}- and γ_{μ} paraproteinemia but only in 4 out of 79 patients with these types of paraprotein. Both the results obtained by Brittin et al. and the present results agree that the intranuclear inclusion bodies in these groups of paraproteinemia are P.A.S. negative.

Earlier investigations seem to indicate that the immunoglobulins are formed in the ergastoplasm of the plasma cells. (cf. Thiéry (54)). By immuno-fluorescence technic it has been shown (8, 48) that also nuclear inclusion bodies in plasma cells contain paraprotein of the same type as the cytoplasm. Furthermore, Brittin et al. (8) presented suggestive evidence that protein other than nucleoprotein is actually synthesized in the nucleus. These investigations may indicate that an intranuclear protein synthesis can occur. It is striking that plasma cells with intranuclear inclusion bodies, when present, only occur in a minority (less than 1 %) of the plasma cells. Therefore, any possible intranuclear paraprotein synthesis, in the form mentioned, although interesting in principle, could well be negligible from a quantitative point of view. It is also possible that the intranuclear bodies have something to do with an abnormal protein metabolism in the cell. A simultaneous occurrence of heavy eosinophilic masses of cytoplasm and naked structureless nuclei is seen occasionally. This may suggest that the cytologic characteristics of the γ_{1-A} type of myelomatosis are the results of cellular changes which finally result in the death of the γ_{1-A} plasma cell. This would be analogous with the conditions in experimental amyloidosis, where the pyroninophilic proliferative phase is followed by a phase with cellular destruction and amyloid formation (53). Only more detailed investigations can elucidate these questions.

Acta Medica Scandinavica Vol. 175, fasc. 5, 1964

From the Geriatric Unit, Old Peoples Town, Copenhagen, Denmark

Coagulability of the Blood in Aged Subjects on Different Diets

By

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The aetiology and pathogenesis of atheromatosis as well as of arterial thrombosis must be considered complex. Among the factors reckoned to be of decisive importance are disturbances of lipid metabolism, especially hypercholesterolaemia. Thus, direct injury to the arterial wall with subsequent deposition of cholesterol in the intima has been considered an early stage of atheromatosis. In recent years, however, the influence of the lipids upon the coagulation of the blood and upon fibrinolysis has been considered a decisive factor in the aetiology of atheromatosis and especially in the formation of thrombi. Foods with a high content of saturated fatty acids are believed to accelerate coagulation and inhibit fibrinolysis, while fats with a high content of poly-unsaturated fatty acids are believed to exert the opposite effect.

Investigations by Geill et al. (5) and by From Hansen et al. (4) have shown that partial substitution of the butter fat contained in the food by certain vegetable oils (corn oil, soya bean oil) materially reduces the incidence of thrombosis in elderly hospital patients. In order to throw further light on the direct cause of these phenomena investigations into the coagulation of the blood were carried out by Ollendorff et al. (7) but these investigations did not give consistent results (*vide* *inf.*).

As also stated in the above-mentioned paper, a number of the methods which have previously been used for evaluating the coagulation of the blood are not well-suited for the purpose. Moreover, the experimental periods in previous studies have been rather short with one exception (Dayton et al. (3). Both circumstances may well explain the marked discrepancy in the literature in respect to the influence of diet upon blood clotting. Cotchett et al. ( ) using the same methods as the present authors in the above-mentioned and in the present study demonstrated hypercoagulability after administration of unsaturated as well as of saturated fatty acids. Most interest attaches to the studies of Buzina et al. (1) who found a pro-

Submitted for publication October 21, 1963.

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hypocoagulability after administration of seal oil. Figs. 1 and 2 show the curves representing the mean values during the two named experimental periods. On the other hand, the curves before and after administration of soya bean oil were identical.

In fig 3 curve *a* represents the mean for all the lipaemic samples during the first period on normal hospital diet and curve *b* the mean during the subsequent period with plenty of butter fat. It may be seen that the high-fat diet induces slight hypercoagulability as indicated by a higher maximum thrombin content. On the contrary curve *c* representing the normal period following the butter fat period, demonstrates an evident hypocoagulability as compared with curve *b* but also though to a lesser extent, when compared with curve from the first normal period This hypocoagulability following butter fat-induced hypercoagulability might be a rebound phenomenon.

In order to elucidate this problem, the mean values were determined in the first and in the second half of the normal period following upon the high-butter fat period. The two curves are plotted in fig 4 The rebound phenomenon is very pronounced in curve 1 which shows marked hypocoagulability In the second half of the same period the rebound phenomenon has disappeared, curve 2 corresponding to those representing other periods on normal hospital diet.

During periods when the patients received the vegetable oil diet nd the marine oil diet, the thrombin generation test showed hypocoagulability as compared with the intervening normal period. No rebound phenomenon was observed.

Fig 5 demonstrates the mean curves for the three periods on a high-fat diet. There is a definite difference between the hypercoagulability in the butter fat period and the hypocoagulability in the vegetable oil period as well as the marine oil period.

Figs. 6 and 7 illustrate the findings during the butter fat period and the marine oil period, when both are divided

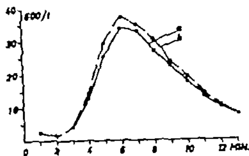


Fig. 1 Thrombin generation test (mean values)) fasting, b) after administration of cream.

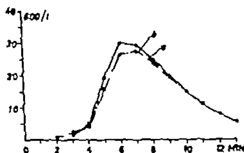


Fig. 2 Thrombin generation test (mean values)) fasting, b) after administration of soya bean oil.

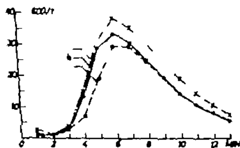


Fig 3. Thrombin generation test (mean values).) normal hospital diet, b) high butter fat diet,) normal hospital diet

Table I. Length of the various periods (on normal diet, butter fat, vegetable oil, and marine oil diet), number of patients and tests

		No. of pat.	Diets	Investigations
	1962			
I	30/4–4/6	24	Normal	3
II	6/6–11/7	24	Butter fat	3
III	30/7–20/8	23	Normal	2
IV	24/8–5/10	23	Vegetable oil	3
V	15/10–9/11	22	Normal	3
	1963			
VI	7/1–16/1	20	Normal	2
VII	24/1–22/2	19	Marine oil	3
VIII	27/2–12/3	19	Normal	2

No blood samples were taken in the period 10/11 1962–6/1 1963.

longed clotting time of whole blood in patients who had been kept on a vegetable oil diet (soya bean oil) for 6 months.

Methods

The following methods were used:

Thrombin generation test (Pitney and Dacie's method in the modification of Ollendorff).

Thromboplastin activation test (Astrup and Ollendorff's method).

Quick's prothrombin-time test using human brain (prepared according to Owren's method).

Serum cholesterol (Carr and Drekter's method).

These methods have been described in detail by Ollendorff et al. (7).

Material

Originally the material comprised 24 patients from one of the nursing departments of the geriatric hospital ("De Gamles By") in Copenhagen. During the period of the study which was nearly one year 5 patients died. The youngest patient was 73 and the oldest 92 years of age. Periods with normal diet alternated with diets rich in butter fat, vegetable oils (soya bean oil, cotton seed oil) and marine oils (fish oil, seal oil) respectively. Table I demonstrates the duration of the different periods. The normal period was 5 weeks, the butter fat period 5 weeks, the vegetable oil period 6 weeks, and the marine oil period 4 weeks.

The normal hospital diet has a fat content of approximately 80 g, mostly in the form of butter fat and margarine, and a caloric content of 2 000–2,500.

In the vegetable oil period butter and ordinary margarine were replaced by a factory made medical margarine containing 65 % unhydrogenated cotton seed oil, and milk was replaced by soya bean oil emulsified in skimmed milk.

During the marine oil period only fish, mostly fat fish like salmon, eel, herring, mackerel, and fat Greenlandic halibut (Hippoglossus vulgaris) were given for dinner and supper instead of meat. Furthermore a highly refined seal oil (iodine value approx. 150) was used partly for cooking and partly for a hospital-made margarine with 80 % unhydrogenated seal oil. This seal oil margarine had very little taste or smell, and the patients accepted it without protesting.

Blood samples from each patient were studied 2 or 3 times in each period, on different days. On these days, fasting blood samples were drawn. Then, the patients were given about 70 g butter fat (cream with butter milk), soya bean oil, or seal oil, respectively and a new blood sample was drawn 5 hours later. A total of 858 blood samples were obtained, i.e. 37 from each of the patients who completed the study period. Thrombin generation tests and thromboplastin activation tests were done on all the blood samples. Moreover the prothrombin time and the serum cholesterol value were determined in the last sample of each period.

Results

Thrombin generation test

There was no really definite difference between fasting and lipaemic samples, but perhaps a tendency to hypercoagulability after administration of cream and to

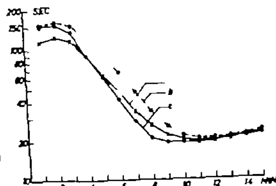


Fig. 8. Thromboplastin activation test (mean values).) normal hospital diet, b) marine oil diet,) normal hospital diet.

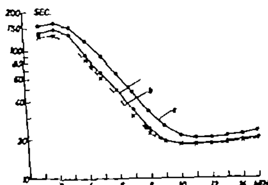


Fig. 9 Thromboplastin activation test (mean alues)) butter fat diet, b) egetable oil diet,) marine oil diet.

no increase during the butter fat diet and only slight decrease (bout 9 %) on vegetable oil and marine oil diets.

Discussion

Thus, the present investigation showed that according to the modified thrombin generation test the patients showed signs of mild hypercoagulability on a diet high in butter fat, while during periods on a vegetable oil diet and on a marine oil diet they showed slight hypocoagulability On the other hand, there was no significant difference between blood samples drawn before breakfast and samples drawn during the lipaemic phases of the two first mentioned diets. This is in keeping with the findings of Lewis (6) Schmidt and Clifford (8) and others.

Table II Mean values of serum cholesterol during the various periods

	Serum cholesterol (mg/100 ml)
I Normal diet	244
II Butter fat	244
III Normal diet	244
IV Vegetable oil	223
V Normal diet	237
VI Normal diet	229
VII Marine oil	222
VIII Normal diet.	246

A factor of particular interest is th *rebound phenomenon* after the butter fat peri od. It manifested itself as distinct hypo coagulability during the first half of th

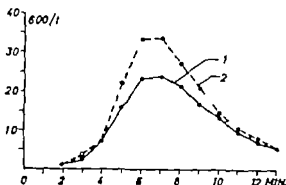


Fig 4 Thrombin generation test (mean values) 1) first half of the normal period following upon the high butter fat period, 2) second half of the normal period following upon the high butter fat period.

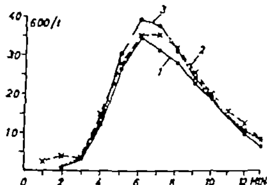


Fig 6. Thrombin generation test (mean values). Butter fat period. 1) first 10-day sub-period, 2) second 10-day sub-period, 3) third 10-day sub-period.

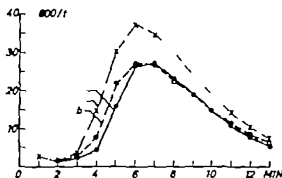


Fig. 5 Thrombin generation test (mean values) a) butter fat period, b) vegetable oil period, c) marine oil period.

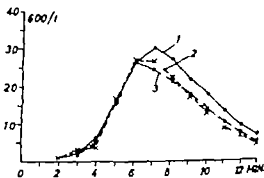


Fig 7 Thrombin generation test (mean values). Marine oil period. 1) first 10-day sub-period, 2) second 10-day sub-period, 3) third 10-day sub-period.

into 10-day sub-periods. The curves, which indicate the mean values for these sub-periods reveal that the maximum effect was not obtained until the last sub-period. Thus, a further effect might have been obtained if the dietary periods concerned had been longer

Thromboplastin activation test

This test gave results similar to those of the thrombin generation test, but the differences were not so marked Fig 8 illustrates the curves during the marine oil period as well as the preceding and succeeding normal periods. During the marine oil period there was slight hypocoagulability In fig 9 the curves representing the three high-fat periods are compared There is no definite difference between the curves for the butter fat and vegetable oil periods while the curve representing the marine oil period shows slight hypocoagulability Thus, the thromboplastin activation test does not appear to be as suitable as the thrombin generation test in these investigations. It was, indeed devised for other purposes.

Determination of the *Quick time* gave no data of interest.

Table II gives the mean values for *serum cholesterol* during the various dietary periods. The variations are slight, there being

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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On a Swedish Family with 51 Members Affected by von Willebrand's Disease

By

Jörgen Silwer and Inga Marie Nilsson

In 1926 and 1931 von Willebrand (28, 29) gave the first description of a haemorrhagic diathesis, occurring in the Åland Islands (Finland). He described the disease as being inherited as a Mendelian dominant, affecting both sexes, and characterized by a prolonged bleeding time, normal coagulation time and normal platelet count. Since von Willebrand's publication a variety of cases resembling those of von Willebrand have been reported by other authors under a variety of names but it is clear from these reports that no agreement had been achieved as to whether the underlying defect in von Willebrand's disease is a qualitative platelet defect or a vascular defect, or both, or whether the defect is different in different patients (4, 21).

In Sweden special interest has been focused on von Willebrand's disease (15, 16, 17, 18, 19, 21). Nilsson and coworkers define von Willebrand's disease as an autosomal dominant inherited haemorrhagic disorder characterized by prolonged bleeding time and AHF deficiency (f. VIII). This definition of von Willebrand's disease has been adopted by several other workers (5, 7, 10, 14 a. o.). The underlying mechanisms of this disorder have been investigated by Nilsson and coworkers. They have found it possible to correct the AHF deficiency, the prolonged bleeding time and the capillary bleedings by injection of human fraction I-0 prepared by the glycine method of Blombäck and Blombäck (2) from normal or haemophilic plasma. Their findings indicate that the primary defect in this disease is lack of a plasma anti bleeding factor (vascular factor) and that this plasma factor correcting the bleeding time is also concerned with the production or activation of AHF. These findings have been confirmed by Cornu et al. (7, 8, 9) and by van Creveld and Mochtar (10).

To date, 86 Swedish patients from 50 families have been examined (18). In not more than in 6 families has a more detailed hereditary investigation been performed. The largest pedigree reported in the literature of von Willebrand's disease is

Submitted for publication October 31, 1963.

subsequent normal period while in its latter half it had disappeared. A similar rebound phenomenon was not observed during the normal periods following upon the vegetable oil and marine oil periods. In a previous study (7) the present authors did not observe this effect of a high butter fat and high vegetable oil diet. The former had no influence at all upon the result of the thrombin generation test, presumably because at that time the diet was not nearly as high in fat as this time — with the large supplement of cream. But in the previous study unlike the present the high vegetable oil diet resulted in slight hypercoagulability. It is difficult to find the explanation. The quantity of vegetable oil was then considerably lower than now. Moreover the periods on unsaturated fatty acids (vegetable oil and marine oil respectively) were longer in the present than in the previous study.

There was no definite relationship between the serum cholesterol value and the coagulability (table II) which is in keeping with the report of Buzina et al (1).

Summary

In a series of 24 patients in the nursing department of a geriatric hospital the influence of the diet upon the coagulation of the blood was studied through one year. The results were assessed on the basis of the thrombin generation test and the thromboplastin activation test.

No difference was found between fasting and lipaemic blood samples after administration of butter fat soya bean oil and seal oil respectively. On the other hand there might be slight hypercoagulability during periods on a high butter fat diet. During the subsequent period on an ordinary hospital diet, the patients developed a distinct hypocoagulability — indicating a rebound phenomenon.

Hypercoagulability was demonstrated in the periods on vegetable oil diet as well as on marine oil diet, but there was no rebound during the subsequent periods on hospital diet.

Acknowledgements

Our sincere thanks are due to the Danish Soya Cake Factory and its staff, especially Mr S. B. Lintz Christensen Civil engineer Director and Mr A. Hvolby Civil engineer Head of the Research Laboratory for producing and supplying us with a highly purified seal oil for the dietary experiments. Our thanks go also to Mrs. D W[illegible], Dietician, and to Mr Borge Dalgård, Staff nurse for their help in performing the study. Aided by a grant from the Danish State Research Foundation.

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II 4 female born 1899

This woman had since early childhood had nose bleeding, gingival bleeding and ready bruisability. Tooth extractions had been followed by prolonged bleeding. Severe bleeding had also occurred after surgical treatment of an abscess of the throat. The bleeding symptoms had been most pronounced during adolescence and had substantially abated with advancing age. The menstrual flow had not been especially profuse. She had 8 children, but no abnormal bleeding had occurred in connection with the partus.

II 5, female born 1903

She had been troubled by prolonged nose bleeding and gingival bleeding in childhood. During adolescence tooth extractions had been followed by prolonged bleeding, which lasted for several days. In the last few years she had had several teeth extracted without abnormal bleeding. She bruised easily. The menstrual flow had been profuse and she had almost always been anaemic. After an abortion in 1937 she bled profusely and the haemoglobin value fell to 7.8 g/100 ml. At the age of 50 hysterectomy was done because of profuse uterine bleeding. The operation was followed by bleeding, and the haemoglobin value fell to 7.4 g/100 ml. Blood transfusions were given. She had 2 daughters. After the last partus the haemoglobin value was 5.8 g/100 ml.

II 6, male born 1903

A twin brother of II 5. H died soon after birth.

II 7 male born 1906

This man had often been troubled by prolonged nose bleeding lasting for days. He had had prolonged bleeding following trauma and tooth extractions. H had never sought medical advice for his bleeding symptoms. The bleeding tendency had decreased with increasing age.

II 8, male born 1908

This man had had symptoms similar to those in the foregoing case. The last year he had been admitted to hospital because of gastrointestinal bleeding. He received 2½ l of blood. Roentgen examination of the oesophagus, stomach, duodenum and colon revealed no pathological changes. In 1962 he had a fatal accident.

II 9 female born 1912

This woman had since childhood had prolonged nose bleeding, ready bruisability and prolonged bleeding following trauma and tooth extractions. The menstrual flow was normal. No immediate abnormal bleeding occurred after her first parturition in 1955 but three weeks later she was admitted to hospital because of profuse uterine bleeding. The haemoglobin level was 8.6 g/100 ml. The bleeding stopped after removal of fragments of membranes and placenta. The second child was delivered by caesarean section. There was no abnormal bleeding.

III 1 male born 1911

No bleeding symptoms.

III 2, male born 1915

H had been troubled by nose bleeding in childhood. H also had gingival bleeding. During the last year tooth extractions had been followed by prolonged and profuse bleeding. After appendectomy 1955 a large haematoma developed in the operative field.

III 3, female, born 1917

This woman was troubled by profuse menstrual flow which had increased the last years. Gynaecologic examination revealed no abnormalities. Otherwise she had no bleeding symptoms. She had borne two children without abnormal bleeding.

III 4 female born 1923

This woman had been troubled by ready bruisability, gingival bleeding, prolonged nose bleeding and prolonged bleeding following trauma. Tooth extractions had been followed by profuse bleeding for which she had to be admitted to hospital. The menstrual flow was profuse and had made her anaemic. Repeated curettage failed to produce any demonstrable effect. The operative specimen was of normal appearance. She had 3 children. No abnormal bleeding had occurred in connection with parturition.

III 5, female born 1919

No bleeding symptoms.

the original family of Åland (11) One hundred and thirty two members of the last 5 generations were known to have had bleeding symptoms. Of those members still living 211 had been examined and the bleeding disorder had been found in 106

In August 1961 a 3-year-old boy was admitted to hospital in Kristianstad because of severe and prolonged bleeding after a cut of the lip Coagulation studies revealed von Willebrand's disease. Inquiry into the familial history revealed that bleeding symptoms were common among relatives of the boy's maternal great-grandfather who belonged to a well-defined and numerous branch of the family In fact it turned out to be the largest family in Sweden with von Willebrand's disease having 51 affected members Many of these have been examined at hospital because of their symptoms, but the type of bleeding disorder had not been recognized Since it was possible to make a thorough investigation of the history of the disorder and laboratory studies of the coagulation factors in 79 members of the family a fairly detailed report of the findings appeared legitimate. Some genetic aspects of the disease are also discussed

Material and methods

Human fraction I O Human fraction I-O containing AHF was prepared at the Chemistry Department II Karolinska Institutet, Stockholm, by the glycine method of Blombäck and Blombäck (2 3) One dose of fraction I-O is prepared from 1400 to 1600 ml of fresh normal plasma

Coagulation tests All the methods used for collection and preparation of the blood samples and for determination of the different coagulation factors have been described elsewhere (23) The *AHF activity* of plasma was assessed by its normalizing effect on the recalcification time of haemophilia A plasma (19, 24) and the amount of AHF present expressed in per cent of that found for a normal standard consisting of a pooled plasma from 10 normal individuals. The *bleeding time* was determined by (1) the method of Duke using standardized haemolets (Dade Reagent, Inc. Miami, Florida U S.A.) Determinations were performed on both ears. (Normal range 1 to 4 minutes.) (2) the method of Ivy as modified by Borchgrevink and Waaler (6) Cuts were made with a surgical blade (Gilette Surgical Blade E), each blade being used for one examination only The bleeding times of 3 transversal standard cuts, 1 mm deep and 10–14 mm long were measured and the mean of the three values noted was taken as the bleeding time of the patient. The overall mean for such triplicate determinations in 35 normal individuals was found to be 9½ minutes (range 5–15½)

Case reports

I 1 male, born 1873

According to his children this man had often been troubled by prolonged nose bleeding He died at the age of 77 from heart stroke

I 2 female, born 1868

She was not known to have had an increased bleeding tendency She died at the age of 50 because of Spanish influenza.

II 1 female born 1888

This woman had been troubled by prolonged nose bleeding and prolonged bleeding following trauma and tooth extractions. No coagulation studies have been performed.

II 2 female, born 1891

This woman had since childhood been troubled by an increased bleeding tendency similar in type to that of her younger sisters. After an abortion in 1922 she had profuse and prolonged uterine bleeding and simultaneous nose bleeding The haemoglobin value decreased to 10 % according to the method of Sahli and the erythrocytes to 960,000/mm³ She died at the age of 69 from myocardial infarction

II 3, male born 1894

No bleeding symptoms.

presumptive diagnosis of thrombasthenia had been made.

The bleeding symptoms had markedly improved in recent years.

III 20, female born 1923

This woman had since childhood been troubled by ready bruisability nose bleeding and increased bleeding after cuts. In 1941 pulmonary tuberculosis and lung haemorrhages ere diagnosed. Therapeutic pneumothorax was repeatedly complicated by haemorrhages. I 1947 she died in association with bleeding after a thoracocentesis. On investigation in 1942 she had a bleeding time of 8 minutes, while the platelet count and the coagulation time were normal.

III 21 male born 1926

He had been troubled by prolonged nose bleeding, which had lasted for 1–2 days. A tooth extraction had been followed by 2 days bleeding

III 22, female, born 1928

This woman said that she bruised readily and had an increased bleeding tendency after trauma and cuts. Otherwise she had no bleeding symptoms. The menstrual flow was normal. She had 3 children but there had been no increased bleeding in connection with parturition.

III 23, female born 1930

In 1948 dental surgery was followed by an abscess of the jaw The abscess was punctured with large gauge needle and the patient bled for several hours. The bleeding stopped spontaneously. Otherwise no bleeding symptoms.

III 24 male born 1935

This man had since childhood been troubled by ready bruisability prolonged nose bleeding and increased bleeding tendency after cuts and tooth extractions.

III 5, female born 1937

This woman had had prolonged nose bleeding, gingival bleeding, haematomas and prolonged bleeding following trauma and cuts. The nasal cavity had been cauterized on several occasions. Extraction of deciduous teeth had been complicated by 1–2 days' bleeding I 1951 haemarthrosis of the lef knee joint developed after puncture because of hydrops. In 1954 she again had got a large bleeding, this time in the right knee joint after puncture of the knee because of hydrops. The haemoglobin level then fell from 11.2 g/100 ml to 7 g/100 ml. On that occasion it was found that the patient had a prolonged bleeding time, namely 10 minutes. The platelet count and coagulation tim were normal. No persistent joint deformities had developed. The menstrual flow was normal.

III 26, female born 1940

This woman had had severe nose bleeding, ready bruisability and increased bleeding tendency after cuts. After a tooth extraction she had bled for several days. The menstrual flow was rather profuse and she had had episodes of anaemia.

III 27 female, born 1930

She had since childhood been troubled by ready bruisability prolonged nose bleeding, gingival bleeding and increased bleeding after cuts and trauma. The menstrual flow was normal. She had 2 children. The first delivery was not followed by undue bleeding. After the second parturition she had profuse and prolonged bleeding, namely from a large vaginal rupture. The rupture was sutured, but she continued to bleed for a week. She received 6 blood transfusions.

III 28, female, born 1939

This woman had symptoms similar to those of her sister (III 27) A tooth extraction had been followed by profuse bleeding lasting for day The menstrual flow was rather profuse. She had 2 children, but there had been no abnormal bleeding in connection with parturition.

III 29, female, born 1929

No bleeding symptoms.

III 30, male, born 1931

No bleeding symptoms.

III 31 female born 1932

She had since childhood been troubled by gingival bleeding nose bleeding and prolonged bleeding after cuts. Tooth extractions had been followed by profuse bleeding lasting for at least day The menstrual flow was normal.

III 6 female born 1921

She often had nose bleeding, usually lasting 5–10 hours, and she bruised readily. The menstrual flow was profuse and prolonged and had made admission to hospital necessary. Excision of a biopsy specimen from the portio was followed by severe bleeding and the haemoglobin level fell to 9 g/100 ml. She had 2 children. The first parturition was followed by prolonged bleeding lasting for about 3 months. The second delivery was normal.

III 7 female born 1923

This women said that she bruised rather readily and sometime bled from the gingiva. No nose bleeding or bleeding after tooth extractions. During an attack of proctitis she had bled from the rectum. The menstrual flow was normal, and delivery of her son was uncomplicated.

III 8 female born 1924

She had been troubled by bleeding from haemorrhoids, but she had not become anaemic. On 3 occasions she had been admitted to hospital because of profuse menstruation. Diagnostic biopsy on 2 occasions had revealed polyps of the corpus and cervix on the first occasion and erosion of the portio on the second. Otherwise she had no bleeding symptoms. She had 2 children and delivery had been uncomplicated on both occasions.

III 9 male, born 1926

He had sometimes had episodes of prolonged nose bleeding. On 2 occasions tooth extraction had been followed by heavy bleeding that lasted the whole night and had to be controlled with a tampon by his dentist.

III 10, male born 1928

He had not observed any increased bleeding tendency. No teeth had been extracted.

III 11 male born 1929

No bleeding symptoms.

III 12, female born 1932

She had had no nose bleeding or gingival bleeding, but thought she bruised readily. After two complicated tooth extractions at which the jawbone was fractured she had bled for 3–4 days. A laparotomy had not been complicated by bleeding. The menstrual flow was prolonged, but not profuse. The delivery of her child had been uncomplicated.

III 13, female born 1936

She bruised readily but otherwise she had not observed any increased bleeding tendency. The menstrual flow was normal. No tooth extractions or other surgical procedures had been performed. She had 3 children, but parturition had never been accompanied by abnormal bleeding. Nor had an abortion been complicated by undue haemorrhage.

III 14 female born 1927

The menstrual flow was rather profuse, but otherwise she had had no bleeding symptoms whatsoever.

III 15, female born 1930

No bleeding symptoms.

III 16 female born 1931

The menstrual flow was sometimes rather profuse, but otherwise she had no bleeding symptoms.

III 17 female born 1933

No bleeding symptoms.

III 18 male born 1941

No bleeding symptoms.

III 19 female born 1918

This woman had since early childhood been troubled by ready bruisability, prolonged nose bleeding, gingival bleeding and increased bleeding tendency after cuts and trauma. Tooth extractions had been followed by profuse bleeding and had required admission to hospital. Thoracocentesis in 1942 was followed by profuse bleeding. On that occasion a prolonged bleeding time (> 18 min.) was observed. The platelet count and the coagulation time were normal. Operation on the nose in 1953 was also followed by abnormal bleeding. The menstrual flow was profuse and often anaemizing. In 1950 the menstrual flow was so profuse that the haemoglobin value decreased to 4.9 g/ml. She then received blood transfusions. She had borne 4 children. At the first 2 deliveries she lost 1200 ml and 650 ml of blood. It had been observed that the bleeding time became normal during pregnancy. A

tractions had been uncomplicated. Otherwise she had no bleeding symptoms. The menstrual flow was normal.

IV 7 male, born 1949

This boy was troubled by prolonged nose bleeding for which he had on several occasions been admitted to hospital. Tooth extractions had been followed by prolonged bleeding. He also had gingival bleeding. At the age of 6 years he was operated upon because of appendicitis. His record sheet contains no note of any increased bleeding tendency.

IV 8, 9 and 10

No bleeding symptoms.

IV 11 female, born 1945

The menstrual flow was rather profuse but otherwise she had no bleeding symptoms whatsoever.

IV 12, female born 1948

This girl had since early childhood been troubled by ready bruisability prolonged nose bleeding, gingival bleeding and increased bleeding after cuts and trauma. Loss of deciduous teeth was often followed by profuse bleeding, which was controlled by gingival sutures.

IV 13, male born 1951

He had had some haematomas but otherwise no bleeding symptoms.

IV 14 15, 17 19 20 21 22 and 23

No bleeding symptoms.

IV 16, male born 1951

He had had episodes of prolonged nose bleeding but otherwise no bleeding symptoms.

IV 18, female born 1956

This girl bruised readily but otherwise she had had no bleeding symptoms.

IV 4 female born 1958

Extraction of deciduous teeth had been followed by severe bleeding for about 3 days. The bleeding had been stopped by local treatment by her dentist. Otherwise no bleeding symptoms had been noted.

IV 25, 26, 27 28, 29 and 30

No bleeding symptoms.

IV 31 male born 1940

No increased bleeding had been noted. The patient had never undergone any surgical operation, and he had not had any of his teeth extracted.

IV 32 female, born 1943

The menstrual flow was rather profuse, but otherwise she had no bleeding symptoms.

IV 33, male, born 1948

This boy had had bleeding symptoms consisting of easy bruisability prolonged bleeding following trauma and cuts. Tooth extractions had been followed by prolonged bleeding.

IV 34 male, born 1947

This boy had gingival bleeding, but otherwise no bleeding symptoms.

IV 35, 36, 37 and 38

No bleeding symptoms.

IV 39 male, born 1959

This boy had often had episodes of severe nose bleeding lasting for days. He also had gingival bleeding, and on one occasion it was so profuse that he was admitted to hospital. The haemoglobin level then had decreased to 8.0 g/100 ml. He bruised readily.

IV 40 41 42, 43 and 45

No bleeding symptoms.

IV 44 male born 1958

This boy had had several episodes of prolonged nose bleeding.

IV 46, male born 1958

This boy bruised readily and he often was anaemic.

IV 47 48, 49 50, 51 52 53 55, 56, 57 58 and 59

No bleeding symptoms.

IV 54 male born 1954

Died few days after birth from congenital heart disease.

She had borne 4 children. After the second delivery she bled 1100 ml.

III 32 male born 1934

This man had been troubled by episodes of prolonged nose bleeding for which the nasal cavity had been cauterized on various occasions.

III 33, male born 1937

This patient had been troubled by episodes of prolonged nose bleeding and during childhood he had on several occasions been admitted to hospital because of severe nose bleeding with haemoglobin levels ranging between 4.8 and 7.2 g/100 ml. He had also had bleeding from the gums, and he bruised readily. Three teeth had been extracted and on every occasion he had had severe bleeding which could not be controlled without suturing or tamponade. At the age of 15 he was operated upon because of acute appendicitis. No bleeding complications were reported. This patient was given two test doses of fraction I-O which corrected the prolonged Ivy bleeding time and increased the AHF level from 30 to 3 % of normal

III 34 female born 1940

This patient had also bruised readily and she had a tendency to nose bleeding gingival bleeding and prolonged bleeding following trauma and tooth extraction. The menstrual flow was profuse and caused anaemia. Curettage on three occasions produced no effect on the profuseness of the menstrual flow. She had received 2 blood transfusions because of the menstrual bleeding. She had 3 children. Th bleeding tendency decreased during the pregnancies. No abnormal bleeding had occurred in connection with the deliveries.

III 35, male born 1932

During military service he had on period of prolonged nose bleeding for which he did not seek medical advice. He had a tendency to bleed from the gums when cleaning his teeth. Tooth extraction was not followed by abnormal bleeding. He had not any other bleeding symptoms.

III 36, female, born 1934

This woman was troubled by gingival bleeding. The menstrual flow was normal. She had 2 children. At the first delivery she lost 900 ml of blood and the haemoglobin level decreased to 8.3 g/100 ml. No blood transfusions were given. The second parturition was not complicated by abnormal bleeding.

III 37 female born 1935

No bleeding symptoms.

III 38, male born 1946

This boy had not observed any increased bleeding tendency. He had never undergone any surgical operation, and he had never had any teeth extracted.

III 39 female born 1948

This girl had had prolonged nose bleeding and gingival bleeding. After extraction of a deciduous tooth she had bled profusely for at least a day. Adenoidectomy in 1960 had also been followed by prolonged bleeding, and the haemoglobin level decreased to 10.8 g/100 ml. A bleeding disorder was then suspected, but the bleeding time, the coagulation time and the platelet count were found to be normal.

III 40 female born 1943

This woman complained of ready bruisability and had had episodes of nose bleeding in childhood. Tooth extractions had also been followed by increased bleeding. The menstrual flow was profuse, for which reason she had often been admitted to hospital. On investigation in May 1962 she was pregnant, and she then had a normal bleeding time and normal AHF content. The delivery was normal. It has not been possible to reinvestigate the patient after parturition.

III 41 female born 19..5

This girl bruised readily. She had had several deciduous teeth extracted, but only one of the extractions had been followed by prolonged bleeding. Tonsillectomy had not been complicated by bleeding.

IV 1 2 4 5 and 6

No bleeding symptoms.

IV 3, female born 1943

Extraction of a deciduous tooth had been followed by copious bleeding that was controlled by suturing the gum. Other tooth ex

Table I. Coagulation studies in the family

Case			Type of disease	Month/year of invest.	Bleeding time (min)			AHF (f VIII) % of normal	B-factor (f IX) % of normal	P&P (Prothrombin + f VII) %	Factor V % of normal	Fibrinogen g/100 ml
					Duke		Ivy					
Coordinate no.	Sex	Year of birth			Right ear	Left ear						
II 3	[illegible]	1894	Normal	10/62	1	1	12	87	128	100	178	0.48
II 4	♀	1899	Mild	5/62	8	6	>30	43	100	94	123	0.31
II 5	♀	1903	Mild	5/62	2	1	>30	47	90	89	146	0.30
II 7	[illegible]	1906	Mild	10/61	2	4	25	38	110	90	60	0.42
				5/62			>30	42	90	94	152	—
II 8	♂	1908 †1962	Mild	5/62	2	2	~30	30	100	99	117	0.39
II 9	♀	1912	Mild	5/62	15	24	>30	29	123	106	106	0.44
III 1	♂	1911	Normal	5/62	1	1	9	82	148	77	92	0.33
III 2	♂	1913	Mild	10/62	3	3	30	48	98	98	128	0.30
III 3	♀	1917	Mild	5/62	4	2	>30	75(?)	118	85	125	0.42
				11/62	2		30	56	110	112	130	0.38
III 4	♀	1923	Mild	5/62	5	3	>30	36	90	89	141	0.27
III 5	♀	1919	Normal	1/63	5	3	12	143	94	97	190	0.35
III 6	♀	1921	Mild	1/63	3	7	>30	30	110	101	148	0.42
III 7	♀	1923	Normal	1/63	7	4	9	112	87	105	140	0.19
III 8	♀	1924	Normal	1/63	4	3	9	91	125	72	133	0.31
III 9	♂	1926	Mild	10/62	3	4	>30	36	85	132	128	0.21
III 10	♂	1928	Mild	11/62	3	4	26	49	123	91	118	0.29
III 11	♂	1929	Normal	10/62	3	5	17	85	163	108	148	0.23
III 12	♀	1932	Normal	2/63	1	3	—	75	73	92	171	0.32
				4/63			15	82				
III 13	♀	1936	Mild	11/62	3	3	28	45	118	86	79	0.28
III 14	♀	1927	Normal	10/62	3	3	15	71	118	118	127	0.29
III 15	♀	1930	Normal	11/62	2	1	13	68	105	90	49	0.22
III 16	♀	1931	Normal	10/62	1	4	20	126	153	132	118	0.47
III 17	[illegible]	1933	Normal	11/62	3	—	11	73	90	79	47	0.22
III 18	♂	1941	Normal	11/62	4	1	9	86	83	84	70	0.22
III 19	♀	1918	Mild	5/62	5	9	26	58	155	95	190	0.44
III 21	♂	1926	Mild	5 62	3	5	23	49	93	76	79	0.54
III 22	♀	1928	Mild	5/6	4	—	25	53	90	101	74	0.29
				10/62	—	—		56	150	104	102	0.3[illegible]
III 23	[illegible]	1930	Normal	5/62	4	4	11	92	83	73	119	0.3[illegible]
III 4	[illegible]	1935	Mild	11/62	14	6	17 30	28	84	78	100	0.3[illegible]
III 25	[illegible]	1937	Mild	5/62	>30	10	>30	30	80	71	85	0.2[illegible]
III 26	♀	1940	Mild	5/62	9	9	>30	43	88	79	92	0.3[illegible]
III 27	♀	1930	Mild	10/62	3	3	>30	42	80	140	125	0.3[illegible]
III 28	♀	1939	Mild	10/62	2	3	>30	29	108	101	100	0.3[illegible]
III 29	[illegible]	1929	Normal	11/62	1	2	17	119	95	93	135	0.3[illegible]
III 30	♂	1931	Normal	11/62	2	3	9	64	148	90	137	0.2[illegible]
III 31	♀	1932	Mild	11/62	4	3	21	44	88	89	82	0.2[illegible]
III 32	♂	1934	Mild	11/62	5	4	>30	41	100	87	98	0.[illegible]
III 33	♂	1937	Mild	12/61	3	2	24	38	95	56	124	
				1/62	4	2	28	30				

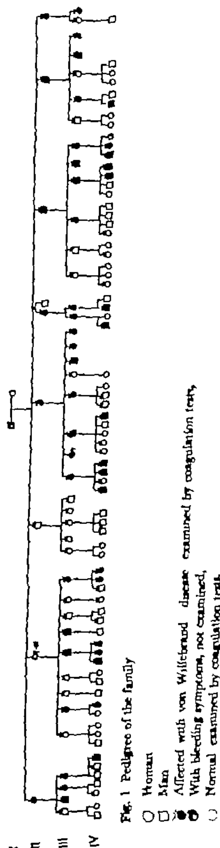


Fig. 1 Pedigree of the family
○ Woman
□ Man
◐/■● Affected with von Willebrand disease examined by coagulation tests.
With bleeding symptoms, not examined.
○ Normal examined by coagulation tests.

IV 60 male, born 1960

This boy had had profuse bleeding from a small cut in the lip and on one occasion after he had bitten his tongue. Otherwise no bleeding symptoms had been noted.

IV 61 male born 1958

This boy was always covered with haematomas. He often had epistaxis and had often been admitted to hospital for cauterisation of the nasal cavity. In August 1961 he was admitted to hospital because of profuse bleeding from a small cut in the lip. The haemoglobin value had decreased to 8 g/100 ml. A prolonged bleeding time was then noted while the platelet count was normal. This boy was then sent to hospital in Malmö for coagulation analysis, which revealed von Willebrand's disease.

IV 62, female born 1960

This girl bruised readily and even trivial scratches bled profusely.

IV 63, female born 1962

This girl had had several episodes of nose bleeding.

IV 64 65 67 68 69 70 and 71

No bleeding symptoms.

IV 66, male born 1955

This boy bruised readily and after a tooth extraction he had had prolonged bleeding for which he had to be admitted to hospital.

Results

The pedigree of the family is given in fig. 1. The results of the coagulation studies on the 79 individuals investigated are summarized in table I. In 4 of the smallest children only the bleeding time was determined (VI 51, IV 58, IV 60, IV 62).

It is clear from table I that 45 of the 75 members investigated completely were found to have decreased plasma AHF activity with AHF values ranging from 16 % to 60 % of normal. The AHF values varied only slightly in those members who were tested on more than one occasion (II 7, III 22, III 33) with the exception of III 3 in whom the AHF value

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Case: Coordinate no.	Sex	Year of birth	Type of disease	Month/year of invest.	Bleeding time (min) Duke: Right ear	Duke: Left ear	Ivy	AHF (f. VIII) % of normal	B-factor (f. IX) % of normal	P&P (Prothrombin + f. VII) %	Factor V % of normal	Fibrinogen g/100 ml
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II 5	♀	1903	Mild	5/62	*2*	*1*	>30	*47*	90	89	146	0.30
II 7	o	1906	Mild	10/61	*2*	*4*	25	*38*	110	90	60	0.42
				3/62			>30	*42*	90	94	152	—
II 8	o	1908 †1962	Mild	5/62	*2*	*2*	>30	*30*	100	99	117	0.39
II 9	♀	1912	Mild	5/62	*15*	*24*	>30	*29*	123	106	106	0.44
III 1	♂	1911	Normal	5/62	1	1	9	82	148	77	92	0.33
III 2	♂	1913	Mild	10/62	*3*	3	30	*48*	98	98	128	0.30
III 3	♀	1917	Mild	5/62	*4*	2	>30	75(?)	118	95	125	0.42
				11/62	*2*		30	*56*	110	112	130	0.38
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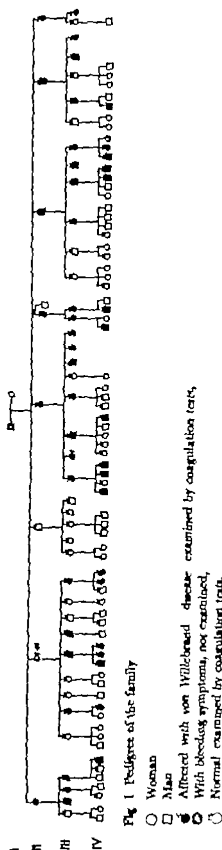


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III 25		1937	Mild	5/62	>30	10	>30	30	80	71	85	0.29
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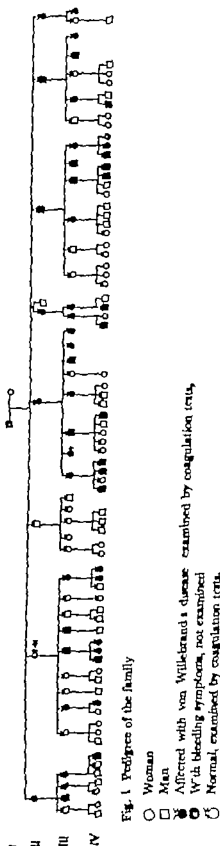


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Normal, examined by coagulation tests.

IV 60 male born 1960

This boy had had profuse bleeding from a small cut in the lip and on one occasion after he had bitten his tongue. Otherwise no bleeding symptoms had been noted.

IV 61 male born 1958

This boy was always covered with haematomas. He often had epistaxis and had often been admitted to hospital for cauterization of the nasal cavity. In August 1961 he was admitted to hospital because of profuse bleeding from a small cut in the lip. The haemoglobin value had decreased to 8 g/100 ml. A prolonged bleeding time was then noted while the platelet count was normal. This boy was then sent to hospital in Malmö for coagulation analysis, which revealed von Willebrand's disease.

IV 62, female born 1960

This girl bruised readily and even trivial scratches bled profusely.

IV 63, female born 1962

This girl had had several episodes of nose bleeding.

IV 64 65, 67 68, 69 70 and 71

No bleeding symptoms.

IV 66 male born 1955

This boy bruised readily and after a tooth extraction he had had prolonged bleeding for which he had to be admitted to hospital.

Results

The pedigree of the family is given in fig. 1. The results of the coagulation studies on the 79 individuals investigated are summarized in table I. In 4 of the smallest children only the bleeding time was determined (VI 51, IV 58, IV 60, IV 62).

It is clear from table I that 45 of the 75 members investigated completely were found to have decreased plasma AHF activity with AHF values ranging from 16 to 60 % of normal. The AHF values varied only slightly in those members who were tested on more than one occasion (II 7, III 22, III 33) with the exception of III 3 in whom the AHF value

Table II. Correlation between bleeding symptoms, bleeding time and AHF level

Case													Bleeding time		
Coordinate no.	Sex	Nose bleeding	Gingival bleeding	Haematomas	Gastro-intestinal bleeding	Prolonged external bleeding following cuts and trauma	Bleeding after tooth extraction	Abnormal bleeding after surgery	Profuse menstruation	Abnormal bleeding after delivery	Abnormal bleeding after abortion	Other bleeding episodes	Duke	Ivy	AHF (f. VIII)
II 1	♀	+				+	+								
II 2	♀	+									+++				
II 3	♂												N	N	N
II 4	♀	++	+	+		+	+	++					+	+++	++
II 5	♀	+	+	+			++	++	+	++	++		N	+++	++
II 7	♂	+				+							N	++	++
II 8	♂				++		++						N	+++	++
II 9	♀	+				+	+			++			++	+++	++
III 1	♂												N	N	N
III 2	♂	+					++	+					N	++	++
III 3	♀								+				N	+++	+
III 4	♀			+			++		++				+	+++	++
III 5	♀												N	N	N
III 6	♀	+		+				++	++	+			N	+++	++
III 7	♀			+	+								N	N	N
III 8	♀				(+)				+				N	N	N
III 9	♂	+					++						N	+++	++
III 10	♂												N	++	++
III 11	♂												N	+	N
III 12	♀						(+)		+				N	N	N
III 13	♀			+									N	++	++
III 14	♀								+				N	N	N
III 15	♀												N	N	N
III 16	×								+				N	+	N
III 17													N	N	N
III 18	♂												N	N	N
III 19		++	+	+		+	++	++	+++	++			+	++	+
III 20	×	+		+		+	++	+++				Lung bleed.	+		
III 21	♂	+					++						N	++	++
III 22						+							N	++	+
III 23								(+)					N	N	N
III 24	♂	+		+		+	+						+	++	++
III 25		++	+	+		+	++						++	+++	++
III 26				+		+	++		+			Joint bleed.	+	+++	++
III 27		+	++	+		+				+++			N	+++	++
III 28			+	+		+	+		+				N	+++	++
III 29													N	+	N
III 30	♂												N	N	N
III 31		+	+			+	+			+			N	+	++
III 32	♂	+											N	+++	++
III 33	♂	++		+			++						N	++	++
III 34	×		+	+		+	++		+++				+	+++	++
III 35	♂	+	+										N	N	N
III 36			+										N	++	++
III 37										++			N	+	N
III 38	♂												N	+++	++
III 39		+	+				++	++					N	++	++
III 40		+		+			+		+				N	N	N
III 41				+			+						N	+++	+

The mean values of bleeding time in right and left ear < 5 min = N, 6—10 min = +; > 10 min = ++;
30 min = +++; ≥ 15 mm = N, 16—24 mm = +; 25—30 mm = ++; > 30 mm = +++;
60 % = N, 30—60 % = +; < 30 % = ++ × Pregnant.

Table I (cont.)

Case: Coordinate no.	Case: Sex	Case: Year of birth	Type of disease	Month/year of invest.	Bleeding time (min): Duke, Right ear	Bleeding time (min): Duke, Left ear	Bleeding time (min): Ivy	AHF (f. VIII) % of normal	B-factor (f. IX) % of normal	P & P (Prothrombin + f VII) %	Factor V % of normal	Fibrinogen g/100 ml
III 34	♀	1940	Mild	1/10/61	8	2	—	52	—	100	95	0.36
			grav.mens.VI	10/10/61	23	10	—	43	—	—	—	—
				5/62	1	8	>30	35	103	66	85	0.25
III:35	○	1932	Normal	4/63	1	3	14	83	70	128	115	0.27
III:36	♀	1934	Mild	5/62	4	3	30	36	110	120	95	0.26
III 37	♀	1935	Normal	11/62	3	4	18	104	—	105	129	0.33
III 38	♂	1946	Mild	11/62	3	2	>30	44	93	66	111	0.25
III:39	♀	1948	Mild	5/62	3	1	27	41	85	68	97	0.24
III 40	♀	1943	Normal	5/62	3	3	7	121	65	65	99	0.29
			grav.mens.II									
III 41	♀	1953	Mild	11/62	2	3	>30	55	130	77	134	0.27
IV 5	♀	1943	Normal	11/62	2	1	16	80	110	73	111	0.29
IV:6	○	1944	Normal	5/62	3	3	8	91	145	92	78	0.23
IV 7	○	1949	Mild	5/62		8	25	48	65	73	89	0.39
IV 12	♀	1948	Severe	1/63	>30	—	>30	34	83	108	180	0.30
IV 16	♂	1951	Mild	10/62	2	3	>30	53	93	132	163	0.31
IV 17	♀	1952	Mild	10/62	1	2	>30	37	80	110	151	0.26
IV 18	♀	1956	Mild	10/62	9	9	>30	31	115	184	168	—
IV:19	♂	1950	Normal	11/62	2	1	13	75	95	88	79	0.26
IV:20	♂	1948	Normal	10/62	2	2	12	114	75	96	142	0.31
IV 22	♀	1950	Normal	2/63	3	4	10	120	90	95	127	0.33
				2/63			12	114				
IV 23	○	1956	Normal	11/62	1	3	12	63	90	78	55	0.33
IV 24	♀	1958	Mild	11/62	3	9	—	38	93	80	78	0.51
IV 25	♀	1959	Mild	11/62	2	1	—	56	100	73	53	0.24
IV 31	○	1940	Mild	11/62	3	3	21	43	108	74	135	0.29
IV 32	♀	1943	Normal	11/62	2	2	11	67	103	82	96	0.24
IV 33	♂	1945	Mild	5/62	3	3	25	54	98	68	92	0.29
IV 34	○	1947	Mild	11/62	3	3	25	45	93	69	89	0.30
IV 35	♀	1950	Normal	10/62	2	2	19	82	135	95	163	0.32
IV 36	♀	1952	Normal	10/62	3	3	10–26	71	70	140	150	0.36
IV:39	♂	1959	Severe	3/63	>30	—	—	49	43	99	145	0.52
IV:40	○	1947	Normal	10/62	2	3	13	81	110	73	96	0.25
IV 41	♂	1950	Normal	10/62	2	2	12	73	65	112	145	0.26
IV 44	♂	1958	Mild	10/62	4	—	>30	20	80	105	153	0.25
IV 46	♂	1958	Mild	10/62	4	—	>15	29	113	144	175	0.27
IV 48	♀	1950	Normal	11/62	1	3	11	104	128	116	97	0.34
IV 51	♀	1958	Normal	11/62	3							
IV 53	♀	1952	Normal	11/62	2	2	15	78	100	86	94	0.27
IV 58	♂	1956	Normal	11/62	2	1						
IV:59	○	1957	Mild	11/62	3	3	20	60	120	104	96	0.26
IV 60	♂	1960	Mild	11/6[illegible]			>30					
IV 61	♂	1958	Mild	10/62	8	15	—	21	98	55	88	0.26
				10/62	9	8	—	16	114			
IV 62	♀	1960	Mild	11/62	8							
IV 66	♂	1955	Mild	5/62	4	5	>30	32	63	94	124	0.40

of whom 116 are still living. Of these who have died, 4 had typical bleeding symptoms and 2 of them had been examined before death. Altogether 51 (23 males and 28 females) of the members of the family had had bleeding symptoms. We have examined 79 members and 46 were found to have von Willebrand's disease.

The examination included 6 of the 9 members belonging to the second generation. We examined all 41 members of the third generation, of whom 40 are still living. The deceased member (III 20) of this generation was a female, who had died 16 years previously and in whom bleeding symptoms had been known and confirmed at examination in hospital. Of the 71 members of the fourth generation, 33 were examined. In most of the cases the bleeding symptoms reported were checked by examination of their hospital record sheets or by inquiry among the doctors who had treated them earlier. As many as 7 of the 9 members of the second generation had a history of bleeding symptoms, and all 7 had children with a tendency to bleeding. II 6 died immediately after birth. In 5 of the 6 studied, the Ivy bleeding time was prolonged and the AHF decreased. The children of II 3, the member who had a normal bleeding time and normal AHF had no bleeding symptoms.

Twenty-one of the 41 members of the third generation had a tendency to bleeding in their history. Laboratory studies in these 41 cases revealed prolonged Ivy bleeding time and decreased AHF in 24.

Of the 71 members of the fourth generation, 11 had a definite, and 4 a possible, tendency to bleeding in their histories. Laboratory studies showed a prolonged bleeding time and decreased AHF in 17 members of this generation and a prolonged bleeding time in 2 children in whom the AHF level was not determined. Some of the members in this generation have not yet reached the age at which bleeding symptoms usually begin to appear.

As pointed out in the introduction, von Willebrand's disease is transmitted by an autosomal dominant character of varying expressivity. This family fulfils several criteria for such a mode of inheritance. Each affected member had one affected parent, and normal offspring of affected members had only normal children. In several cases the disease could be followed through 3 consecutive generations. The condition was equally common in both sexes.

An observation difficult to explain in this family is that the number of affected members was far too high, i.e. in generations II and III more than the expected half of the children of affected parents. As to the second generation, it is possible that not only the father (I 1) but also the mother (I 2) was a carrier. But, as far as we know, the mother had no symptoms of any increased bleeding tendency. I 1 was a foundling, and there is no reason to suppose that I 1 and I 2 were blood relations. The over-representation of affected members in the fourth generation was less marked.

The severity of the disease and symptoms varied widely, not only among the affected members but also among affected sibs. In this respect von Willebrand's disease differs in a characteristic way from mild haemophilia A, for example, where all affected members of a given family have the same level of AHF and the same severity of bleeding symptoms (23). Some of the members of the present family had decreased AHF values and prolonged Ivy bleeding times but deny any symptoms of increased bleeding tendency (III 10,

was 75 % at the first examination and at the second 56 % (the bleeding time was prolonged on both occasions) In III 34 AHF values were higher during pregnancy than afterwards. Of the members low in AHF 25 had an Ivy bleeding time of more than 30 minutes and 20 an Ivy bleeding time ranging between 19 and 30 minutes. The Duke bleeding time was markedly prolonged in only 4 patients (II 9 III 25 IV 12 IV 39) In the other patients it was normal or only slightly prolonged

In the 30 individuals with normal plasma AHF activity the AHF values ranged between 63 % and 143 % of normal. All these members had normal Duke bleeding times The Ivy bleeding time was normal except in 7 members (III 11 III 16 III 29 III 37 IV 3 IV 35 IV 36) in whom it was slightly prolonged and ranged between 16 and 26 minutes The AHF levels in these 7 members were 85 126 119 104 80 82 and 71 % respectively They had no bleeding symptoms

The members investigated had B factor values ranging from 63 to 163 % of normal except for IV 39 (a 4-year-old boy) who had 43 % The values found for prothrombin + factor VII (P & P) were essentially normal in all the members, the values ranging from 55 to 184 % of normal. The values found for factor V were also largely normal the values ranging from 53 to 190 % of normal except in III 15 and III 17 who had only 40 and 47 % (these members had normal AHF values and no bleeding symptoms) The fibrinogen values ranged between 0 19 to 0 54 g/100 ml.

The coagulation time, the one-stage prothrombin time and the recalcification time of plasma which are not included in table I were within normal ranges in all the cases studied. The patients did not show any signs of pathologically increased fibrinolytic activity The platelet count was determined in only a few patients, but in several of the patients the platelets had been counted on earlier hospitalization and then found to be normal.

Blood group analysis (Dr O Ramgren) were performed in 67 members of the family (40 affected members and 27 normal members) in respect to A B, O anti-D anti C, anti E, anti-c, anti-e, anti-M anti N anti K anti k, anti Fy^a and anti Fy^b No correlation was found between the various blood groups and the gene or genes responsible for the disease.

In table II the bleeding symptoms of the various patients are classified according to severity as + ++ and +++ The symbol +++ is to be understood as repeatedly severe profuse bleeding requiring hospitalization and intense blood therapy (or when no transfusions were given the bleeding caused a verified severe blood loss) With ++ we mean severe bleeding also requiring hospitalization or medical consultation but not always blood therapy With + we mean abnormal bleeding not requiring hospitalization or blood therapy

Discussion

It is clear that the clinical and laboratory findings in the affected members of this family are compatible with a diagnosis of von Willebrand's disease. One of the members (III 33) was also given human fraction I-O which corrected the prolonged Ivy bleeding time and increased the AHF level

The first member known to have had bleeding symptoms was I 1 (born in 1873) who died some 20 years ago. It is his offspring that constitute the present material, which consisted of as many as 123 members (55 males and 68 females)

9 caesarean section was not attended by any undue loss of blood, possibly because the AHF is normally increased during pregnancy (20).

In 2 sisters with tuberculosis (III 18 and III 19) pleural puncture for maintenance of pneumothorax and for tapping of pleural exudate was accompanied by bleeding from the pleura. In III 19 the bleeding was fatal. But the bleeding was only a contributory cause of death, for the pulmonary tuberculosis was very advanced, and the patient was in a very poor condition.

Finally many of the members of the family reported ready bruisability and several of them also had haematomas at the time of the examination. But no large intramuscular haematomas were ever reported.

Summing up, the bleeding symptoms in this family were usually mild, sometimes moderate, and rarely life-threatening. Apart from the above-mentioned member (III 19) the bleedings had never been fatal or shortened life. Most of the male members were manual labourers and the condition had hardly interfered with their work. The most troublesome bleedings were those of the mucosae of the nasal cavity, oral cavity and the female genital organs. The symptoms reported by the females were on the whole more severe than those described by the males.

Many had been admitted to hospital because of their bleeding episodes, and examination on admission had often included determination of the bleeding time, coagulation time and platelet count. The coagulation time and the platelet count had always proved normal. The Duke bleeding time was prolonged in some of the severe cases but normal in others, even in association with obstinate bleeding.

It is obvious that the routine tests used in the investigation of increased bleeding tendency, i.e. coagulation time, Duke bleeding time, platelet count and one-stage prothrombin time, are not sufficient for tracing cases of von Willebrand's disease. Nilsson et al. (22) compared the Duke and Ivy bleeding time in von Willebrand's disease and other haemorrhagic disorders. In agreement with other authors (9, 25) they found Ivy's method to be much more sensitive than Duke's method. In several cases of mild von Willebrand's disease the Duke bleeding time was normal or only slightly prolonged while the Ivy bleeding time was definitely prolonged in all these cases. Therefore supplementation of the Duke bleeding time with determination of the Ivy bleeding time in routine investigations will considerably enhance the possibilities of discovering mild cases of von Willebrand's disease. Patients with prolonged Ivy time and normal platelet counts can then be referred to special laboratories for determination of AHF and other coagulation factors.

As to the treatment of the bleeding symptoms in this family, the latter could usually be controlled by relatively simple conventional methods varying with the site of the bleeding. But blood transfusions were often necessary. Specific treatment is now available for von Willebrand's disease, namely fraction I-O (17).

It is important that patients and the physicians consulted be well acquainted with the disease and know that measures should be taken in the management of severe bleeding episodes and in connection with surgery. For this reason all known patients with von Willebrand's disease have received identification cards containing data on their blood group, disease, nature and severity and those meas-

III 38 IV 17 IV 26 IV 54) while other members with roughly the same AHF level and the same prolongation of the bleeding time had had troublesome bleeding symptoms.

Most of the affected members reported that the bleeding symptoms were most troublesome during childhood and adolescence. In middle age and later the pathologic tendency had practically disappeared Similar observations have been made by other investigators (1 11 12, 13 26 27)

The commonest bleeding symptom is probably epistaxis It was prolonged and often so troublesome that the patients sought medical advice. Only in one patient had the loss of blood been excessive (III 33)

Gingival bleeding had often been observed, but usually only after brushing of the teeth. In several cases, however the gums bled spontaneously mostly in girls and young women and then usually at night A 4-year-old boy (IV 39) was admitted to hospital with severe and prolonged gingival bleeding and a haemoglobin value of 8 g/100 ml.

Profuse and prolonged bleeding after tooth extractions was one of the commonest symptoms Control of such bleeding usually required suturing or tamponade.

Only one of the patients had had severe gastrointestinal bleeding (II 7)

A common finding in the females was that the menstrual flow was profuse. Thus, 8 of 16 affected members of reproductive age reported profuse menstrual flow as against 5 of 13 unaffected members of the same family It is not always easy to decide whether menstruation in a given case should be regarded as profuse or not. However several of the affected members of this family had been admitted to gynaecologic departments because of the bleeding and several of them also had anaemia. Curettage had as a rule been performed but it had never produced any demonstrable effect. In one patient (III 34) the menstrual flow was so profuse as to cause shock and make blood transfusions necessary

Increased bleeding after parturition had occurred in 7 of the affected women with children (table II) These 7 women had also borne children without excessive loss of blood at parturition Seven of the affected women had not lost any abnormal amount of blood in association with parturition. One (III 27) had a severe and prolonged bleeding episode after delivery but mainly from a rupture of the vagina. She was given 6 blood transfusions.

Heavy bleeding had also occurred in association with abortion. The loss was largest in II 2 in whom the haemoglobin value (Sahli) dropped to 10 %

Joint bleeding was uncommon in this family Only one member (III 25) had had haemarthrosis but without any permanent deformity of the joint.

Prolonged bleeding from trivial scratches and cuts was common. Thus in IV 61 bleeding from a small cut in the lip produced very obstinate bleeding It was this case that prompted the present investigation

Only few of the affected members have been subjected to operations other than tooth extractions. Appendectomy had been performed in III 2 and III 33. In the former case a large haematoma had developed in the operation field but in the latter no bleeding complication occurred. Abnormal bleeding had occurred after surgical treatment of an abscess of the neck, after adenoidectomy and after an operation on the nose in II 4 III 39 and III 19 Hysterectomy in II 5 was complicated by increased bleeding In II

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ures which should be taken in the event of severe bleeding attacks together with addresses and telephone numbers of laboratories from which fraction I-O may be obtained.

Summary

A family with von Willebrand's disease the largest one in Sweden is described. The family consisted of 123 members (55 males and 68 females) belonging to four generations. Of these members 116 are still living Altogether 51 (23 males and 28 females) of the members of the family had had bleeding symptoms. The examination included 79 members, and 46 were found to have von Willebrand's disease. In the second generation 7 of 9 members had the disease. All 41 members of the third generation were examined and 24 of them were found to have prolonged Ivy bleeding time and decreased AHF Of the 71 members of the fourth generation 15 had a tendency to bleeding in their histories. Laboratory studies in 33 cases of this generation revealed prolonged Ivy bleeding time and decreased AHF values in 19 This family fulfils the criteria of an autosomal dominant transmission of the bleeding disorder The penetration of the gene was high. The number of affected members in generations II and III was more than the expected half of the children of affected parents.

The affected members had AHF values ranging from 16 % to 60 % of normal. Of the members low in AHF 25 had an Ivy bleeding time of more than 30 minutes and 20 an Ivy bleeding time ranging between 19 and 30 minutes The Duke bleeding time was markedly prolonged in only 4 patients. In the other patients it was normal or only slightly prolonged Other coagulation factors were normal. The family members with normal AHF values had a normal bleeding time.

The bleeding symptoms in this family were usually mild sometimes moderate, and rarely life-threatening, but the severity of the disease and symptoms varied widely not only among the affected members but also among affected sibs. In most cases there was a good correlation between bleeding symptoms and the coagulation findings. The most troublesome bleedings were those of the mucosa of the nasal cavity oral cavity and the female genital organs. Profuse bleeding after tooth extractions was one of the commonest symptoms. The symptoms reported by the females were on the whole more severe than those described by the males. The bleeding symptoms were most troublesome during childhood and adolescence and virtually disappeared in middle age and later

The bleeding symptoms in this family could usually be controlled by relatively simple conventional methods, but blood transfusions were often necessary

The importance of diagnosing even mild cases of von Willebrand's disease is stressed. Supplementation of the Duke bleeding time with determination of the Ivy bleeding time in routine investigations considerably enhances the possibilities of discovering mild cases of von Willebrand's disease

Acknowledgement

This investigation was supported by grants from the Swedish Medical Research Council and from the National Institutes of Health, Public Health Service (Research Grant H-07066)

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ures which should be taken in the event of severe bleeding attacks, together with addresses and telephone numbers of laboratories from which fraction I O may be obtained

Summary

A family with von Willebrand s disease the largest one in Sweden is described. The family consisted of 123 members (55 males and 68 females) belonging to four generations. Of these members 116 are still living Altogether 51 (23 males and 28 females) of the members of the family had had bleeding symptoms. The examination included 79 members and 46 were found to have von Willebrand s disease. In the second generation 7 of 9 members had the disease. All 41 members of the third generation were examined and 24 of them were found to have prolonged Ivy bleeding time and decreased AHF Of the 71 members of the fourth generation 15 had a tendency to bleeding in their histories. Laboratory studies in 33 *cases of this generation revealed prolonged* Ivy bleeding time and decreased AHF values in 19 This family fulfils the criteria of an autosomal dominant transmission of the bleeding disorder The penetration of the gene was high. The number of affected members in generations II and III was more than the expected half of the children of affected parents.

The affected members had AHF values ranging from 16 % to 60 % of normal Of the members low in AHF 25 had an Ivy bleeding time of more than 30 minutes and 20 an Ivy bleeding time ranging between 19 and 30 minutes. The Duke bleeding time was markedly prolonged in only 4 patients. In the other patients it was normal or only slightly prolonged. Other coagulation factors were normal. The family members with normal AHF values had a normal bleeding time.

The bleeding symptoms in this family were usually mild sometimes moderate, and rarely life threatening but the severity of the disease and symptoms varied widely not only among the affected members *but also among affected sibs. In most* cases there was a good correlation between bleeding symptoms and the coagulation findings. The most troublesome bleedings were those of the mucosa of the nasal cavity oral cavity and the female genital organs. Profuse bleeding after tooth extractions was one of the commonest symptoms The symptoms reported by the females were on the whole more severe than those described by the males. The bleeding symptoms were most troublesome during childhood and adolescence and virtually disappeared in middle age and later

The bleeding symptoms in this family could usually be controlled by relatively simple conventional methods, but blood transfusions were often necessary

The importance of diagnosing even mild cases of von Willebrand s disease is stressed. Supplementation of the Duke bleeding time with determination of the Ivy bleeding time in routine investigations considerably enhances the possibilities of discovering mild cases of von Willebrand s disease

Acknowledgement

This investigation was supported by grants from the Swedish Medical Research Council and from the National Institutes of Health, Public Health Service (Research Grant *H*-07066)

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Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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Serum Haptoglobin Level in Conditions Associated with M-Components

By

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M-components (subgroups Immunologic γ-globulins = γ = γ_{ss} = 7 S γ, β_{2A}-globulins = γ_{A}, β_{2M}-globulins = γ_{1M} = 19 S γ, γ = γ_L = (Bence Jones proteins) micromolecular type) of type γ_{1M} are the most important diagnostic findings in macroglobulinaemia Waldenström (41) but have also been observed in several other conditions (22 24 30, 32, 40 41). Macroglobulinaemia Waldenström is accompanied by anaemia in about 80 per cent of all cases (14 18 22 37 39). This has been ascribed to several factors, such as deficient erythropoiesis, increased destruction of the erythrocytes, external blood losses and iron deficiency (13 18, 22 31 39). Thorough erythrokinetic and red-cell survival studies have been performed in only one small series (13). During the last decade some 15 cases with M-components of type γ_{M} and haemolytic anaemia (1 7 8, 10 13 15 16, 18, 22 26 34 43) have been reported. Electrophoretic γ_1-peaks (10, 11) have also been observed in acquired haemolytic anaemia and those examined were shown to consist of γ_{1M}-globulins (11). In macroglobulinaemia Waldenström several factors may be assumed to contribute to an increased erythrocyte destruction, e. g. increased plasma viscosity (38) high frequency (50 %) of splenomegaly (18 22) and an increased tendency of the red blood cells to aggregate. Some γ_{1M}-globulins, cold agglutinins (16) have also a marked affinity for erythrocytes. In lymphatic leukaemia, with which macroglobulinaemia Waldenström is closely related, haemolyti anaemia is also fairly common.

In an attempt to assess the frequency of markedly increased erythrocyt destruction the serum haptoglobin concentration was measured in 100 patients with M components of type γ_{1M} and for comparison, also in 50 cases with M-components of type γ and 7 S γ respectively

In otherwise uncomplicated haemolytic anaemia (2 3 29) with a turnover of the total erythrocyte cell mass of twice normal or mor (9 27 33) the haptoglobin concentration usually falls to almost zero. In association with inflammatory conditions, however the synthesis rate increases

Submitted for publication October 31 1963.

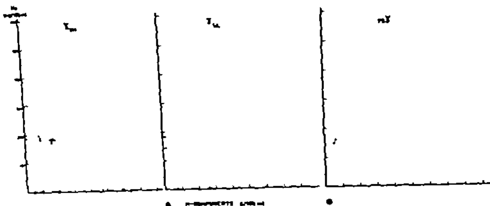


Fig. 2. Serum haptoglobin concentration (Hp) in relation to that of the M-components in patients with M-components of immunologic type a) γ_{2M}, b) γ_{1A}, c) 7 S γ

mitted at our hospital. In one (No. 156) the patient had the clinical picture of macroglobulinaemia Waldenström and both were found to have an acquired haemolytic anaemia with hyperbilirubinaemia, reticulocytosis, increased carbon monoxide haemoglobin concentration, positive Coomb test and high cold agglutinin titre. One patient (No 552) whose clinical picture made the diagnosis of macroglobulinaemia Waldenström most probable, had pronounced splenomegaly. In one case (No 315) the post mortem diagnosis was lymphatic leukaemia but the leucocyte values were always normal. In one case (No. 352) the clinical diagnosis was undetermined. In these 3 cases any haemolytic anaemia was not clinically observed. In 3 (Nos. 70, 204 and 323) the case records were not available (foreigners) but 2 of them had previously been found by Professor Waldenström (41) to have macroglobulinaemia Waldenström.

Of the 2 patients with M-components of type γ_{1A}, one (No 501) had a firm diagnosis of myelomatosis without clinically observed haemolytic anaemia. In the other (No. 321) the patient had severe anaemia of clinically uncertain origin and required repeated blood transfusions. Coomb's test was negative.

The concentrations of haptoglobin in relation to the concentrations of M-components of the 3 different types are given in figs. 2a—c. High haptoglobin values were found only in sera in which the concentration of the M-components was low while low and normal values were found in sera with M-components either low or high in concentration. The frequency of high haptoglobin values decreased with increasing concentration of the M-components, and accordingly the same was true for the mean values.

Scatter diagrams of the concentration of the α_1-globulin and albumin fractions, respectively in relation to the concentration of M-components of the 3 different immunologic types showed an essentially similar distribution to that in figs. 2a—c.

Table I gives the mean concentrations of haptoglobin, α_1-globulin fraction and

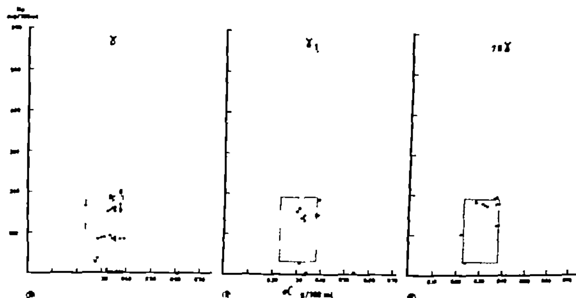


Fig 1 Serum haptoglobin concentration (Hp) in relation to that of the paper-electrophoretic α_1 globulin fraction ( ) in patients with M-components of immunologic type a) γ_{1M}, b) γ_{1A}, c) 7 S γ

(21 28 42) and the haptoglobin concentration may then be normal or elevated despite increased erythrocyte turnover (9) When the erythrocyte turnover is normal the haptoglobin level rises largely in proportion to the degree of inflammation (23 35) If the erythrocyte turnover is simultaneously increased the haptoglobin values will be less increased normal or even decreased (28) It should therefore be possible to form a better opinion of the erythrocyte turnover by relating the haptoglobin concentration to the degree of activity of the disease. As a measure of this we used the concentration of the paper-electrophoretic α_1-globulin fraction whose changes in disease are dominated quantitatively by α_1 antitrypsin and acid α-glycoprotein, both of which increase in the presence of inflammation (19 21 36)

Material and methods

Out of the material described in a previous paper (4) the first 100 cases with M-components of type γ_{1M} for which sera were available, and 50 random cases with M-components of type γ_{1A} and 7 S γ respectively were selected.

The methods and criteria used for electrophoretic and immunologic classification of M-components in serum have been described previously (4)

The serum haptoglobin concentration was determined by a slight modification (25) of Jayle s activation method (20)

The normal range for the paper electrophoretic α_1-globulin fraction is 0.25—0.38 g/100 ml serum, for the albumin fraction 4.2—5 3 g/100 ml serum and for haptoglobin 30—190 mg/100 ml serum measured as haemoglobin binding capacity

Results

The serum haptoglobin concentrations in relation to the concentrations of the α globulin fraction are given in figs. 1a—c.

Of the patients with M-components of type γ_{1M} γ_{1A} and 7 S γ respectively an haptoglobinaemia (< 10 mg/100 ml serum) was found in 8 (8 %) 2 (4 %) and 0 (0 %) respectively

Of the 8 cases with M-components of type γ_{1M} two (Nos. 156 339) were ad

monstrated by Jayle & Boussier (21). In addition, Nyman (28) found a good correlation between the concentration of haptoglobin and that of the paper-electrophoretic α_2-globulin in infections and aseptic necrosis. In cancer, however, Nyman found a poorer correlation (28), the reason for which is not known. An increased or decreased erythrocyte turnover can hardly be the only explanation. Our series, which consists mainly of patients with more or less markedly malignant processes, is more comparable to Nyman's cancer series, and it also shows a low correlation between the concentrations of haptoglobin and of the α_2-globulin fraction (figs. 1a—c).

The fact that some of the patients with a normal haptoglobin concentration had simultaneously an increased concentration of α_2-globulin fraction may suggest an increased erythrocyte turnover in these cases. However, in other cases the haptoglobin concentration was found to be increased despite a normal concentration of the α_2-globulin fraction. This can hardly be explained entirely by an increased external loss of any of the components of the α_2-globulin fraction — at any rate no evidence for such an assumption was observed — or by decreased erythrocyte turnover. It would therefore appear that the increase in the rate of synthesis of haptoglobin is not proportional to that of α_1-antitrypsin and/or acid α_1-glycoprotein in these cases. This limits the value of the concentration of the α_2-globulin fraction as an indicator of the rate of synthesis of haptoglobin and thereby also of the haptoglobin concentration as an indicator of the erythrocyte turnover. This implies that no safe conclusions can be drawn regarding the erythrocyte turnover rate except in the patients with anhaptoglobinaemia, even though several of the other cases may have an increased erythrocyte turnover, especially those with a normal haptoglobin concentration and simultaneously increased concentration of the α_2-globulin fraction. An increase in erythrocyte turnover might, however, not be large enough to be the only cause of the anaemia in the majority of cases with M-components.

As may be seen from table 1 and figs. 2 a—c, both the mean concentration and the range of variation of haptoglobin decrease with increasing concentration of the 3 types of M-components. This might be due to one or more of the following factors: selection of material, changed erythrocyte turnover, changed plasma volume, changed capacity of the organism to react to such inflammatory agents as normally stimulate haptoglobin synthesis.

A possibility that cannot be excluded is that the material was selected in such a way that M-components in low concentrations were incidental findings in patients with some other co-existing disease with a marked inflammatory reaction, while patients with M-components in high concentration sought medical aid for their fundamental disease and that the inflammatory reaction was then less marked. Patients with diseases associated with M-components show a decreased resistance to infection and often have complicating pneumonia (5). It is therefore remarkable that of altogether 33 patients with M-components in a concentration of at least 4 g/100 ml only one had a markedly increased haptoglobin concentration (> 240 mg/100 ml). This also applies to the α_2-globulin fraction whose concentration was markedly increased (> 0.42 g/100 ml) only in 4 of these cases. It is therefore not probable that the lower haptoglobin concentration in patients with M-components in high concentration

Table I The mean haptoglobin concentration (Hp) (mg HbBC/100 ml) and the corresponding mean α globulin concentration (α_1) (g/100 ml) and albumin concentration (alb) (g/100 ml) in sera with M-components grouped according to immunologic type concentration (g/100 ml) and paper-electrophoretic mobility

Immunologic type of M-components		Total	The sera grouped according to					
			Concentration of the M-components			Paper-electrophoretic mobility of the M-components		
			0–1.9	2.0–3.9	> 4.0	$\beta_{2-3}-\gamma$	γ_{1-2}	$\gamma-\gamma$
γ_{1M}	Mean conc. of							
	Hp	165	208	144	110	170	174	154
	α	0.38	0.41	0.38	0.34	0.39	0.38	0.37
	alb	3.6	3.7	3.9	3.1	3 4	3.6	3.8
	No. of sera	100	48	24	28	37	25	38
γ_{1A}	Mean conc. of					$\alpha_2-\beta_{1-2}$	β_2	$\beta_2-\gamma-\gamma_{1-2}$
	Hp	145	200	123	75	138	129	173
	α	0.35	0.38	0.36	0.30	0.33	0.37	0.36
	alb.	3.8	4 0	3.8	3.3	3.8	4.0	3.5
	No. of sera	50	22	16	12	18	17	15
7 S γ	Mean conc. of					$\beta-\gamma_{1-2}$	γ	$\gamma_{1-2}-\gamma$
	Hp	205	233	205	155	201	187	228
	α	0.38	0.42	0.37	0.34	0.38	0.38	0.39
	alb.	3 6	4.1	3.6	2.9	3.5	3.5	3.9
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albumin in patients with M-components varying in concentration paper-electrophoretic mobility and immunologic type.

Discussion

The anhaptoglobinaemia in the 8 patients with M-components of type γ_{1M} suggests that the erythrocyte turnover in these was twice normal or more corresponding to an erythrocyte survival of 60 days or less. At least 2 of the patients were found to have an acquired haemolytic anaemia with a positive Coomb s test and a high cold agglutinin titre.

The anhaptoglobinaemia in the 2 patients with M-components of type γ_{1A} is compatible with an increased erythrocyte turnover

An increased erythrocyte turnover resulting in anhaptoglobinaemia thus appears to be relatively rare in conditions associated with M-components, but may possibly be more common in patients with type γ_{1M} than in patients with the other two types of M-components.

The conclusions that may be drawn concerning the other cases depend on whether factors other than a changed erythrocyte turnover influence the correlation in concentration between haptoglobin and α_1-globulin fraction. A good correlation between the level for haptoglobin and acid α_1-glycoprotein (orosomucoid) in inflammations has been de-

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Acknowledgments

The author wishes to thank Professor Jan Waldenström, M. D. for helpful criticism.

This investigation was supported in part by Public Health Service Research Grant E-4703, from the National Institutes of Health.

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was due mainly to the composition of the material.

In conditions with M-components in high concentration the erythrocyte turn over may be increased but this cannot explain the simultaneous decrease in the concentration of the α_1-globulin and albumin. Of the 10 cases of anhaptoglobinaemia only 4 had *M-components in a* concentration of 4 g/100 ml or more.

An increase of the plasma volume with *increasing* concentration of M-components might explain the decrease in concentration of the haptoglobin as well as of the α_1-globulin fraction and albumin. Björneboe and Jarnum (6) were also able to show experimentally that in hypergammaglobulinaemia the plasma volume increases in proportion to the γ-globulins. Judging from data given by Cline et al. (12, 13) the plasma volume is often increased in conditions with M-components, even though no good correlation could be found with the concentration of the M-components. If however one assumes, as did Bjorneboe and Jarnum (6), that the increase of the plasma volume is secondary to the colloid osmotic effect of the immunoglobulins, it should be most pronounced in the presence of M-components of type 7 S γ and least in the presence of type γ_{1M}. However, we did not find the decrease of the concentration of haptoglobin, α-globulin fraction or albumin to vary with the type of M-components.

Another possible explanation why both the *concentration* of haptoglobin and of the α-globulin fraction decrease when the concentration of the M-components increases is that, in *patients* with M-components in high concentration, the organism is affected to such an extent by the fundamental disease that *it* cannot react normally to such agents as normally stimulate the synthesis of haptoglobin, α_1-antitrypsin and acid α_1-glycoprotein. This would also be compatible with our findings that a markedly increased concentration of haptoglobin and α_1-globulin is so rare in patients with M-components in high concentration (see above).

It is clear from table I that the mean *concentrations of haptoglobin, the* α_1 globulin fraction and albumin do not vary appreciably with the paper-electrophoretic mobility of the M-components.

Heremans (17) has demonstrated the occurrence of complex formation between haptoglobin and M-components, particularly of type γ_{1A}. Our findings suggest that this complex formation either does not result in a decrease of the haptoglobin concentration (e. g. by increased elimination from serum in analogy with what has been found for the haptoglobin-haemoglobin complex) or is rare.

Summary

The serum haptoglobin concentration was determined in 200 patients with diseases associated with M-components of different types: 100 γ_{2M}, 50 γ_{1A} and 50 7 S γ. Anhaptoglobinaemia was found in 8, 2 and 0 cases respectively. The mean concentration of haptoglobin was found to be lower and its range of variation narrower in patients with M-components in high concentration than in those with M-components in low concentration. This also applies to the paper-electrophoretic α-globulin fraction and albumin and may be due to several factors, for example to changed mode of reaction of the organism, to changed plasma volume, and to the composition of material and as far as haptoglobin is concerned to changed erythrocyte turnover. Only a poor correlation was found between the

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

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Disorders of Calcium Metabolism Investigated with Strontium^{85}

By

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The function of the human skeleton is not static. It is composed of a complicated dynamic tissue in which processes of bone formation and resorption are continuously taking place. On the surface of the bone crystals a hydration layer is found in which several elements are present in the ionized state. Calcium is the most important of these elements and it is believed that calcium exchange takes place rapidly between this layer and the surrounding body fluid on the one hand, and by a much slower process, with the interior of the crystal on the other (13). Because of its rapidly mobilizable calcium reserves, skeletal tissue plays an important role in the homeostasis of the calcium level of the plasma and extracellular fluid. The rate of calcium deposition in the bone and the size of the exchangeable calcium pool cannot be measured satisfactorily be means of classical balance studies. For this reason use has been made in recent years of radioactive substances (1, 3, 4, 5, 8, 9). Various isotopes have been employed including calcium^{45}. This isotope, however, has the disadvantage of being a weak beta-emitter which makes it difficult to measure *in vivo* and moreover it also has a long half-life of 152 days. Calcium^{47} is a gamma-emitter with the short half-life of 5 days, which should make it the ideal isotope for this type of investigation but unfortunately it is difficult to produce and it frequently contains a hardly negligible quantity of calcium^{45}. Strontium^{85} has a half life of 65 days and being a strong gamma-emitter is easily measurable *in vivo*. Investigations carried out on rats and humans, by Bauer et al. (2) and Spencer et al. (17) respectively showed that strontium is excreted by the kidney at a rate 3—8 times that of calcium. Skeletal tissue, however, does not appear to discriminate between these two elements. It is possible therefore, to make use of this isotope in the investigation of calcium metabolism in a variety of pathological conditions and in various stages of these diseases, as for example before and after treatment.

Methods and material

In the interpretation of the data obtained from studies with radioactive tracers, it is useful to adopt a certain model, representing the

Submitted for publication October 31, 1963.

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t_0 one is able to trace what would be the theoretical course of the serum concentration during the first two days if it were possible for distribution throughout the pool to take place instantaneously after injection. In this way a serum concentration S_0 is found at t_0. In a similar manner U is obtained from the urine curve. In reality there is of course no immediate distribution throughout the pool; therefore the serum concentrations in the first 48 hours are higher than the theoretical values referred to above and relatively larger quantities are excreted in the urine. For this reason the extrapolated serum values may be obtained theoretically with a dose smaller than the one administered. This corrected dosage (D_c) is found by extrapolating the "rectilinear" section of the retention curve to time t_0 (fig. 3). The retention curve represents the percentage of the injected dosage of radioactive material still present in the organism at any given time, i.e. the injected dose minus the total excretion in the urine and faeces.

The exchangeable calcium space is estimated in litres (equivalent to litres of serum) / body weight by means of the formula

$$E = \frac{D_c}{S_0 \times \text{body weight}}$$

The change in the activity of serum (S) can be represented by the following equation

$$\frac{dS}{dt} = -KS$$

in which the solution $S = S_0 e^{-Kt}$.

K, the rate constant of disappearance of radioactivity from the serum, can be calculated from the slope of the straight section of the serum curve. The urine curve may also be used for this purpose, since it runs parallel to the serum curve. This rate constant K is the sum of various rate constants, determined by the uptake of strontium by the deep bone (k) and the excretion via the urine (k_u) and the faeces (k). The loss of strontium via the faeces was neglected. This excretion had been determined in 16 patients and, after ten days, it was found to amount to no more than 10 % of the injected dose. The constants are calculated as follows

$$K = \frac{0.693}{T^1/_2}$$

$T^1/_2$, the half period, being the time taken for S_0 to fall to half its initial value.

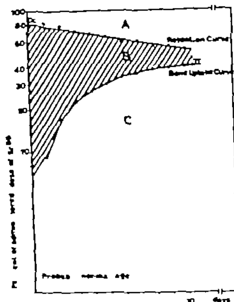


Fig. 3. Behaviour of an intravenously administered tracer-dose of Sr^{85}. For explanation see text.

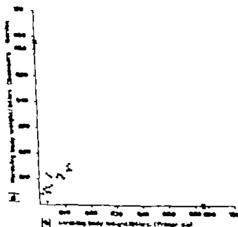


Fig. 4. Comparison of the results calculated according to the methods of Fraser et al. and of Eisenberg and Gordon. It can be seen from this figure that those calculated according to Eisenberg and Gordon are distinctly higher.

$$k_u = \frac{U}{D}$$

$$k = K - k_u$$

The bone accretion rate $B = k_1 E$ in litres/24 hours/kg body weight.

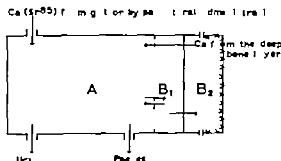


Fig 1 Simplified mod l representing the distribution of stores of body calcium.
Exchangeable calcium pool.
A = Calcium in plasma, extra and intracellular fluid. B_1 = Superficial bone. B_2 = Deep bone.

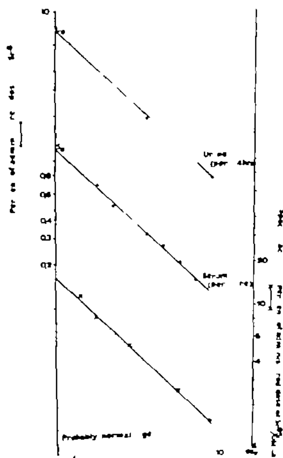


Fig 2 Serum concentrations per litre serum, total urinary excretion per 24 hours (both expressed as a percentage of the administered dose) and serum specific activity are plotted semilogarithmically against time.

various compartments of the body throughout which the administered isotope is distributed. Several studies suggest that body calcium is divided between two pools, a small one which contains the rapidly exchangeable calcium and is composed of several compartments, namely the plasma, extracellular fluid, intracellular fluid and the surface of the bone crystals, and a large pool which contains the less readily available calcium in the deeper layers of the bone (fig 1) Calcium is supplied to the exchangeable pool via the oral or parenteral route and by bone resorption it leaves this pool via the urine and faeces and on being taken up by the deep bone. The uptake by the deep bone comprises the calcium incorporated into newly formed bone as well that received as result of the slow exchange between the surface and the interior of the crystal (14)

During our investigations 20–50 μc of strontium[85] were administered intravenously to each subject. The radioactivity in the serum and urine samples was measured with the aid of a well-type scintillation detector Counts were obtained with a standard error of $\pm 3\%$ except for those specimens taken during the later phases of each study when the standard error was about 6 Calcium levels in the blood and urine were determined with the aid of a flame photometer

Analysis of the data

The data were analysed according to the method described by Fraser et al. (7) It should be stressed that these authors used stable strontium as a tracer The concentration of the radioactivity in the serum and the total daily excretion of radioactive material in the urine (expressed as percentages of the administered dose) were plotted against time on semilogarithmic paper (fig 2) The curves thus obtained are not rectilinear during the first 48 hours and t is assumed that the shape of this portion of the graph is determined not only by the rate of bone uptake and excretion but also by the process of distribution of the injected tracer throughout the exchangeable pool. After 48 hours straight lines are obtained. It may be assumed that during the test period no strontium returns from the deep bone to the exchangeable pool, for if there was a considerable recycling a decrease of the slope of the straight line would occur (9) By extrapolating the straight section of the serum curve to time

weight vary from 0.55 to 0.85 for the exchangeable calcium pool, and from 0.04 to 0.1 for the bone accretion rate. The values for the bone accretion rate, found in our presumably normal subjects, corresponded with these figures.

Fig. 5 represents the results obtained in patients with various non-malignant diseases.

Raised values were found in *thyrotoxicosis* and *Paget's disease* and this tallies well with the data found in the literature.

One case of burnt-out *acromegaly* had normal values. Two cases showed persistently raised values, the one when tested after partial hypophysectomy and the other after hypophyseal implantation of radioactive gold. A fourth acromegalic was treated by irradiation of the pituitary and although there was a significant lowering, the bone accretion rate did not fall to normal (fig. 5)

Of the two patients with *Klinefelter's syndrome* one had normal and the other increased values.

One case of *congenital hypopituitarism*, treated with corticosteroids, had a normal bone accretion rate.

Normal values were obtained in two patients with *hyperparathyroidism*, diagnosis which was confirmed in both cases on the operating table. There was no radiographically diagnosable bone disorder detected in these cases the blood calcium level was very high (24 mg%) in one case and slightly increased in th other The alkaline phosphatase in both cases was slightly abov normal. Fraser et al. (7) found increased values in all their patients with hyperparathyroidism, although those subjects who did not present clinical evidence of bone disease had the least deviation from normal. Eisenberg and Gordon (6) on the other hand, pointed out that in their cases of hyperparathyroidism without histologically demonstrable bone lesions the bone accretion rate was normal the values were increased in all patients with bone involvement regardless of whether this was detectable on X-ray examination or not. Dow and Stanbury (5) also found a normal exchangeable calcium pool in a patient with hyperparathyroidism. They did not estimate the bone accretion rate.

A patient with post-operative hypoparathyroidism, treated with AT 10 showed symptoms of *AT 10 intoxication* The bone accretion rate in this case was slightly increased and it rose markedly during the administration of corticosteroids.

Seven patients with *osteoporosis* were examined, one of whom suffered from osteogenesis imperfecta, one from osteoporosis as a result of prolonged corticosteroid therapy and five from senile osteoporosis. The latter five patients had almost normal bone accretion rates, which is in agreement with the results obtained by other authors in the senile and postmenopausal forms of osteoporosis (7 9) This finding, however does not support the previously generally accepted theory of Albright et al according to which osteoporosis is the result of a reduced formation of bone matrix. Heany and Whedon (9) suggest that bone atrophy n certain forms of osteoporosis is due to an increased bone resorption. Nordin (16) and Harrison et al. (8) have simultaneously reported the results of investigations which suggest that, in a number of forms of osteoporosis, the cause may be a prolonged calcium deficiency in the diet. As a result of the activity of the parathyroid gland which attempts to keep the blood calcium at normal levels, such a deficiency in the diet would lead to an increased bone resorption. Severe cases

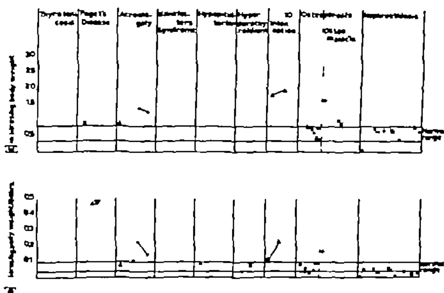


Fig 5. Results of Sr^{85}-test in various non-malignant diseases.

Fig 3 represents the behaviour of an intravenously administered dose of strontium^{85} Curve I is the retention curve and curve II the bone uptake curve. Area A represents the cumulative excretion of strontium^{85} in the urine and faeces, area B the decrease of strontium^{85} in the exchangeable calcium pool, and area C the cumulative uptake of strontium^{85} by the bone.

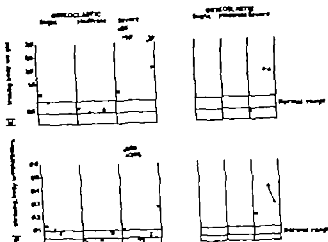
In addition to the above method we also analysed the data obtained in our patients according to the method employed by Heany and Whedon (9) in which use is made of specific activity curves (fig 2) A reasonable degree of agreement was found on comparing the two results. Closer agreement was achieved however when the same data were analysed according to the method used by Eisenberg and Gordon (6) (fig 4) but the values for the bone accretion rate thus obtained were approximately 20 % higher As has been pointed out by these authors, this discrepancy might mainly be due to the fact that in estimating the corrected dose Dc^{85} we assumed as did Fraser and associates the total body retention of strontium to be linear on a semilogarithmic plot this assumption can be shown mathematically to be only approximately true. Eisenberg and Gordon (6) calculated Dc by substracting from the injected dose the difference between the actual and theoretical values of urinary excretion of Sr^{85} during the mixing period. This latter value was obtained by extrapolating the urinary excretion curve to time t = o.

The investigations were carried out on 60 patients. No normal controls were included. Three patients admitted as suffering from possible bone disorders did not show any disturbance in calcium metabolism and were therefore considered normal. The remainder of the patients could be classified as follows invasive bone disease 22 osteoporosis 7 combination of osteomalacia and osteoporosis 2 nephrolithiasis or nephrocalcinosis with or without hypercalciuria 10 thyrotoxicosis 3 Paget disease 3 acromegaly 4 Klinefelter's syndrome 2 hypopituitarism 1 hyperparathyroidism 2 AT 10 intoxication 1

Most patients received for at least one week preceding the investigation a normal hospital diet containing about 1 000 mg Ca and 1 400 mg phosphate daily Some patients, however were on a low calcium diet of about 250 mg Ca and 800 mg phosphate daily During the period of investigation the calcium and phosphate content of the diet were kept constant.

Results and discussion

In view of the fact that no healthy controls were investigated we made use of the normal values provided by Fraser et al (7) According to these authors the normal values, expressed in litres/kg body



. 6. Results of Sr^{85}-test in patients with several bone disease.

Fig. 6 shows the results we obtained in 2 patients with extensive bone disease. According to the X ray and biochemical evidence, eighteen subjects had mainly osteoclastic metastases. There were on the other hand, four cases of osteoblastic metastases from prostatic carcinomata. The patients were further subdivided, in accordance with the radiological findings, into those with mild, with moderate and with severe bone involvement respectively. In the group of the osteoclastic bone secondaries, a normal bone accretion rate was found in the mild and moderate cases (with one exception) but 5 of the 10 patients with extensive bone lesions had markedly increased values. This suggests that in cases with osteoclastic bone secondaries, there may be considerable bone formation in addition to excessive bone resorption. These results, however do not fully agree with those obtained by Fraser et al. (7). In their cases with osteoclastic bone secondaries they found normal values for the exchangeable calcium pool and the bone accretion rate, with only one exception namely case of breast cancer after hypophysectomy.

In our 4 patients with osteoblastic secondaries the bone accretion rate was increased as was to be expected. In one case there was a marked decrease after castration and the administration of oestrogens, and this decrease was accompanied by a very considerable improvement in the clinical condition.

One finds it repeatedly stated in the literature that an increase in the bone accretion rate is, as a rule, accompanied by an increase in the exchangeable calcium pool. Rich et al. (17) using calcium[45] and strontium[85] infusions, went so far as to consider the size of the exchangeable calcium pool a parameter for estimating the bone accretion rate. This relationship can be explained by the fact that the mineral crystals in newly formed bone are more widely spread, so that their surfaces have better contact with the surrounding body fluid. We also found a reasonably close correlation between these two values (fig. 7). The exceptions were mainly cases with bone metastases. This is hardly surprising for however diffuse the process may be, there are always considerable quantities of normal bone present, while

may manifest themselves as osteitis fibrosa. These authors further drew attention to the fact that a typical osteoporosis may arise when calcium absorption by the intestine is disturbed as for example in steatorrhoea It is not clear why in some cases an inadequate calcium supply results in osteoporosis while in others osteomalacia is produced Lichtwitz et al (13) examined the disorders in calcium metabolism found in patients after gastrectomy and on bone biopsy they discovered the histological pictures of both osteomalacia and osteoporosis. Though they do not exclude the possibility that calcium deficiency may be the sole cause of osteoporosis they are of the opinion that an additional protein deficiency may play a role in these cases Bauer et al (3) using P^{32} found a reduced bone accretion rate in one case of idiopathic osteoporosis while more recently Eisenberg and Gordon (6) found reduced values in cases of postmenopausal osteoporosis. The latter authors assume a reduced osteogenesis to be present in this form of osteoporosis

As mentioned above, our five patients with senile osteoporosis had almost normal bone accretion rates, but we did observe a reduced rate in one osteoporotic patient who had been receiving large quantities of corticosteroids for a number of years. The latter finding agrees well with the concept of an inhibition of osteoid formation by the corticosteroids if the bone disorder were due to a corticosteroid induced decrease of calcium absorption from the gut, one should expect either a normal or an increased bone accretion rate. One female patient aged 65 with *osteogenesis imperfecta* had a low normal value for bone formation the defect in the osteoblasts, which is the cause of this disorder had presumably been partly corrected with the passage of time.

Two cases which on clinical examination and bone biopsy were considered to be a combination of *osteoporosis* and *osteomalacia* had an increased rate of bone deposition

Of the ten patients with *nephrolithiasis* we examined two had no hypercalciuria, two had hypercalciuria due to tubular acidosis, and six had an increased calciuria for which no cause could be found. It was surprising to find that five of these patients with essential hypercalciuria showed an abnormally low bone accretion rate. In this connexion it is pertinent to refer to the observations of Lichtwitz et al. (13 14) From among patients with essential hypercalciuria these authors isolated a group with a special biochemical syndrome which they called calcium-diabetes In these patients the hypercalciuria was accompanied by a reduced calcium excretion in the faeces and increased absorption by the intestine. On a low calcium diet or after the administration of sodium phytate there was a marked decrease in the calciuria although it remained higher than normal These authors wonder whether they were dealing here with a syndrome in which the pathological process is situated primarily in the intestinal wall thereby allowing an abnormally high degree of calcium absorption to take place. This results in an increased supply to the kidney and an overloading of the enzyme system which plays a role in the tubular reabsorption of calcium. We investigated one of our patients with this in mind and it was found that on an intake of 1000 mg of calcium per os the excretion of calcium in the faeces was low and absorption via the intestine increased. It is possible that increased intestinal absorption causes an inhibition of parathyroid activity and thus a decreased bone accretion rate

although it is true that this was observed only in patients with normocalcaemia. Our two patients with hyperparathyroidism, on the other hand, had normal values. It therefore appears that investigations with radioactive tracers will not always provide the solution in the sometimes very difficult differential diagnosis of hyperparathyroidism and malignant tumour.

Probably the test will also be of importance in the evaluation of the results of therapy in patients with bone disorders.

Summary

The calcium metabolism was studied in 60 patients by means of a tracer dose of Sr^{85}. In agreement with results quoted in the literature, increased values for the exchangeable calcium pool and the bone accretion rate were found in conditions such as Paget disease, thyrotoxicosis and acromegaly. Two patients with hyperparathyroidism without radiologically diagnosable bone lesions had normal bone accretion rates. Almost normal values were also found in cases of senile osteoporosis. Five of the six patients with essential hypercalciuria had low bone formation rates. A possible explanation for this finding is suggested. Patients suffering from osteoblastic bone metastases showed an increased rate of bone deposition. In cases of osteoclastic secondaries the bone accretion rate was found to be elevated in several patients with a severe degree of bone involvement whereas it was usually normal when the extent of bone invasion was slight or moderate.

The test may be of value in judging the degree of activity of bone disease and in evaluating the effect of therapy.

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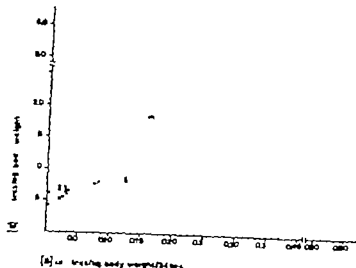


Fig. 7 Correlation between exchangeable calcium pool (E) and bone accretion rate (B)

× Bone metastases.

● Non-malignant diseases.

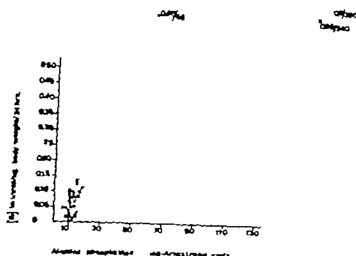


Fig. 8. Correlation between bone accretion rate (B) and alkaline phosphatase.

× Osteoclastic bone metastases.

○ Osteoblastic bone metastases.

● Non-malignant diseases.

an uneven blood distribution undoubtedly exists in those areas affected by the metastases.

The relationship between the bone accretion rate and the alkaline phosphatase in the blood was also investigated but no clear relationship could be discovered (fig. 8). This is contrary to what one might expect, since the value of the alkaline phosphatase in cases with normal liver function is generally considered to be an indication of the degree of osteoblastic activity.

As regards the clinical usefulness of the method described it may be pointed out that the usual diagnostic methods are generally adequate in conditions in which there is an increased bone accretion rate, such as Paget's disease, osteomalacia, thyrotoxicosis and acromegaly. But in these cases the investigation can certainly be of value if one wishes to study the degree of bone involvement. Fraser et al. (7) find the test useful in the differential diagnosis of hypercalcaemia. They found the bone accretion rate increased only in hyperparathyroidism, and not in cases with bone metastases. Several of our patients with osteoclastic bone metastases had an increased bone accretion rate,

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From the Psychiatric Research Centre (Head: Hans Forssman, M. D.) Ulleråker Hospital, University of Uppsala, Ulleråker, Sweden

Positive Sex Chromatin in Men with Epilepsy

By

Gunnar Hambert

Prader et al. (4) found that only 2 out of 9 chromatin-positive boys had normal electroencephalograms. Two of the abnormal records showed the abnormalities characteristic of epilepsy.

Dumermuth (1) studied the electroencephalograms of 14 young males with the Klinefelter syndrome. Only 1 of the 14 had a completely normal curve. 1 showed borderline abnormality and the remaining 12 had abnormal records. Four of the 1[illegible] with abnormal records showed an electroencephalographic pattern typical of epilepsy, with generalized spikes and slow waves or focal sharp waves. On photic stimulation one of the boys got an attack of grand mal for the first time.

During the past two years, I have been screening the male patients in different psychiatric institutions for positive sex chromatin, using the buccal smear method. Whenever positive sex chromatin has been found, I have had the patient tested again and have also given him a thorough clinical examination. Every time the patient was proven chromatin-positive. I have also found him to show hypogonadism, with distinct hypoplasia of the testicles, and often other signs as well. Nine chromatin-positive males with the Klinefelter syndrome were found among the patients at the mental hospital to which our department is attached. 1 of these 9 had epileptic seizures and 1 was definitely known to have had epilepsy before. So far electroencephalograms have been taken in 40 of our chromatin-positive males from the various mental hospitals. In 6 cases the records were normal, in 24 abnormal and in 10 borderline. Further details on the electroencephalograms will be reported in another connection.

As these observations pointed to a causal connection between the Klinefelter syndrome and epilepsy, I decided to examine a series of institutionalized epileptic males, and carried out this investigation in the spring and summer of 1963. In August of 1963 Gerhard Koch (2) pointed out that valuable information might be obtained by screening populations of this kind.

Sweden has six institutions specially for persons with epilepsy which admit male patients. The results of screening the males in these six institutions are seen from

Submitted for publication November 4, 1963.

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positive, and all 4 showed the clinical disorders making up the Klinefelter syndrome. The incidence was thus 0.78 per cent, as against 0.27 per cent in the general male population. Statistically the difference is probably significant. The author discusses the connection between this observation and the observation which he and others have made, that men with positive sex chromatin often have abnormal electroencephalograms. He points out how desirable it would be if populations of epileptic males in other countries were also screened for positive sex chromatin.

Acknowledgements

I wish to extend my sincere thanks to the supervisors and physicians at the six epileptic institutions for their kind cooperation in this study. I am also grateful for the financial support the study received from the insurance company Eir Foundation for Rheumatologic and Psychiatric Research, and from Anton and Dorothea Bexelius and Karl Petrén Memorial Funds.

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Table I Frequency of positive sex chromatin among males in Swedish institutions for the epileptic

Institution	No. examined	No. chromatin-positive
Ervallahemmet	51	2
Fogdarödshemmet	59	0
Margaretahemmet	27	0
Rolngegården	55	0
Stora Sköndal	237	2
Vilhelmsro	83	0
Total	512	4

table I Altogether 512 men and boys were examined except for an occasional patient on leave, these 512 covered all the institutionalized epileptic males in Sweden at the time. Four out of the 512 proved to be chromatin positive, and physical examination revealed the clinical signs of the Klinefelter syndrome as well Thus 1 out of every 128 of these epileptic males had the Klinefelter syndrome, an incidence of 0 78 per cent. Maclean et al. (3) calculated from the data in a combination of several series covering 6 801 unselected newborn males, that 0 27 per cent of the general male population had positive sex chromatin. Thus the incidence in my series was about three times as high as that observed in a general population Though my series is the largest of its kind that can be obtained in this country it is relatively small The difference between the incidence for it and the one Maclean calculated is only probably significant (0 05 > p > 0 025)

Discussion

Thus positive sex chromatin occurred about three times as often in my series of institutionalized epileptic males as in the general male population. Before a causal connection between the epilepsy and this unusually high rate can be assumed the observation must be confirmed by series from other countries. There are not enough institutionalized cases in Sweden alone to allow a definite conclusion. That the high incidence was not due to chance is indicated by the observation that a number of Klinefelter patients in other forms of institution had epilepsy and even more so by the observation that many chromatin positive men have abnormal electroencephalograms.

Koch (2) observed epilepsy in a woman with triple X chromosomes. He pointed out, also that the observations of Prader et al (4) indicated that there might be a connection between the XXy constitution and epilepsy On the other hand he did not believe that, relatively speaking there were many cases of the Klinefelter syndrome among all cases of epilepsy This is borne out to a certain extent by the results in the present study

The possibility of a connection between the Klinefelter syndrome and epilepsy is of great theoretical interest. One can think of several ways in which the two might be connected It may be that the chromosomal aberration also leads to abnormality in the central nervous tissue, or makes it more vulnerable than otherwise. It may be that the deficient testosterone activity causes injury to the cerebrum during a certain phase of development. Finally it may be that the aneuploidy like other forms of embryopathy leads to premature birth resulting in cerebral lesion

Summary

A buccal-smear survey was made of the 512 men in Swedish institutions for the epileptic. Four proved to be chromatin-

positive, and all 4 showed the clinical disorders making up the Klinefelter syndrome. The incidence was thus 0.78 per cent, as against 0.27 per cent in the general male population. Statistically the difference is probably significant. The author discusses the connection between this observation and the observation which he and others have made, that men with positive sex chromatin often have abnormal electroencephalograms. He points out how desirable it would be if populations of epileptic males in other countries were also screened for positive sex chromatin.

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Acta Medica Scandinavica. Vol. 175, fasc. 5 1964

From King Gustaf V Research Institute, Stockholm, Sweden

The Distribution of Intravenously Injected Rheumatoid Factor in the Body of Mice

Preliminary Report

By

NANNA SVARTZ

For the purpose of studying the distribution of the rheumatoid factor in the body of animals the following procedure was used.

1. Purification of the rheumatoid factor (RF) according to the method earlier described (Acta med. scand. *158* 163 1957 & *160* 87 1958)

2. Labelling of RF with Iodine[131] (L.-E. Plantin)

3. Autoradiographic investigations performed at the Ullberg laboratory of the Veterinary School, with the aid of L. E. Appelgren and A. Hansgren. These comprised the following stages a) Intravenous injection of the labelled substance into a series of mice (see Ullberg S. Acta radiologica, Suppl. 118 1954) b) The mice were killed by immersion in a cold liquid (CO_2 or liquid air) at different times after the injections c) Sagittal sections were made through the whole body of the animals. Cellulose tape was put on every section to keep the tissues together and d) The sections were pressed on photographic films and exposed for 3—12 weeks.

Figs. 1—2 show autoradiograms from mice injected with RF labelled with Iodine[131]. It should be added, that the labelled factor had kept its capacity of giving rise to a positive sheep cell test.

The first picture is an autoradiogram showing the distribution of the labelled RF 2 days after injection. The substance shows a pronounced accumulation in the connective tissue everywhere in the body e. g. in the skin, the walls of the vessels, the fasciae and the joint capsules.

There is also a certain affinity for the cartilage, e. g. on the vertebrae. Besides, there is a concentration in the lungs, probably due mainly to the high quantity of blood in the pulmonary tissue. A peculiar radioactivity is present in the whiskers. A strong radioactivity was found in the thyroid gland (fig. 1). According to earlier experience with iodine-labelled substances, this is probably due to liberated iodine. In this connection it should be noted that after intravenous injection of pure iodine, radioactivity is found nearly only in the thyroid gland and stomach and not in the connective tissue.

The findings three days after injection are about the same as in fig. 1 except that the radioactivity is slightly weaker.

Submitted for publication February 24 1964.

Acta Medica Scandinavica. Vol. 175, fasc. 5, 1964

From the Second Medical Department, University of Helsinki (Head Ilmari Vartiainen, M. D)
Helsinki, Finland

The Effect of Intrinsic Factor on the Schilling Test in Fish Tapeworm Carriers

By

I P PALVA

Addition of intrinsic factor (IF) preparation has helped t some extent to raise low Schilling test values obtained in tapeworm pernicious anemia (TPA) (4 5 7 8) There have been conflicting interpretations of this phenomenon. It has been suggested on the one hand that IF disturbance is important in the development of TPA (4 5) and, on the other that tapeworm impairs the absorption of vitamin B_{12} in the host organism even in the presence of IF (8) Absorption of B_{12} is impaired in atrophic gastritis, but this can be remedied by adding IF (2, 15, 16) However gastritis is known to be a common condition in TPA patients (14) Therefore, the present study was undertaken to compare the IF-induced elevation of Schilling values with the incidence of gastritic changes in fish tapeworm carriers

Series and methods

The series consisted of 28 fish tapeworm (*Diphyllobothrium latum*) carriers. Eighty-six specimens were taken from the gastric body mucosa of these patients by Sielaff suction biopsy tube The specimens were fixed in 5 % neutral formalin and stained by the hematoxylin-eosin, hematoxylin-van Gieson and PAS techniques. The Schilling test was performed using 0.6 µg ^{60}Co B_{12} (Abbott Lab., North Chicago, Ill.) as the test dose. One capsule of concentrated IF preparation (Abbott) was given in Schilling test II Student's t-test was employed in the statistical analysis.

Submitted for publication November 8, 1963.

Results

Table I shows the IF induced increase in the Schilling values compared with the morphologic status of the gastric mucosa. In 3 (30 %) of 10 tapeworm carriers with normal gastric mucosa the Schilling value rose to at least double the original value. A corresponding rise occurred in 4 (80 %) out of 5 tapeworm carriers with superficial gastritis and in 11 (85 %) out of 13 patients with atrophic gastritis. The last mentioned figure is significantly ($P < 0.01$) higher than that for the normal group.

Discussion

There are many well-known pointers to the importantance of IF deficiency in the etiology of TPA. For instance, some

In none of the sections was any radioactivity found in the spleen, the lymphatic glands or the bone marrow

Fig 2 demonstrates radioactivity in the joint capsule. Here, a part of section is shown in high magnification. For comparison a neighbouring section, stained with haematoxylin-eosin is shown in fig 3

Summary

After intravenous injection of labelled rheumatoid factor into mice, radioactivity was mainly found in the connective tissue None was found in the spleen, the lymphatic glands or the bone marrow

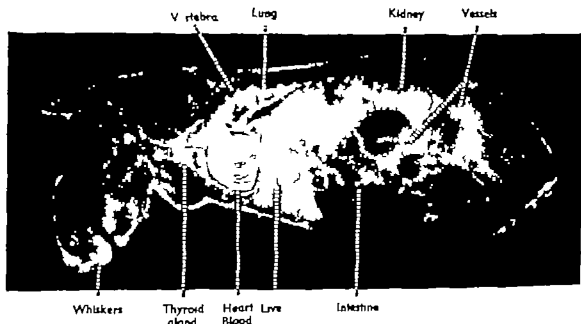


Fig 1 Autoradiogram showing sagittal section of a mouse injected intravenously 2 days earlier with I^{131} labelled heumatoid factor (see text)

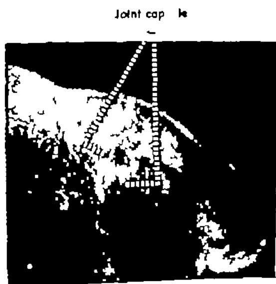


Fig 2. Two days after injection of I^{131}-labelled RF Radioactivity in the joint capsule and also in the subepithelial layer of the skin in the fasciae and the walls of the vessels (high magnification)

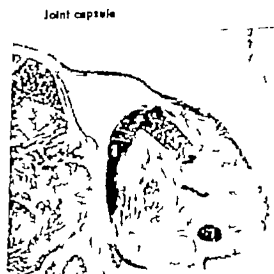


Fig 3 A section adjacent t fig 2 stained by haematoxylin-eosin.

Acknowledgement

Dr. M. Siurala, M. D. kindly performed and interpreted the gastric biopsies.

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Table I The Schilling tests without and with IF compared with the morphologic changes of the gastric mucosa in fish tapeworm carriers

State of the gastric mucosa	Schilling test		Index of increase
	Without IF	With IF	
Normal	8.7	16.8	1.9
	1.0	1.0	1 0
	6.2	8.1	1.3
	3.8	5.4	1 4
	8.0	12.5	1.5
	0.1	4 0	40
	1 6	3.2	2 0
	3.0	4.2	1 4
	7 0	13.0	1.9
	0.4	15.0	37.5
Superficial gastritis	1.5	29 0	19.3
	8.1	24 0	3.0
	1.8	2.6	1.5
	3.9	17.8	4 6
	3.2	14.0	3.4
Atrophic gastritis*	1.5	3.9	2.6
	4.2	15.7	3.7
	5.0	28.0	5.6
	0.6	0.4	0.7
	0.8	6.4	8.0
	0.7	6.0	8.6
	0.5	8.7	17 4
	5.0	4.5	1.0
	2.2	38.7	17.6
	2.5	7.5	3.0
	0	6.2	
	0	3.1	
	8.1	16.2	[illegible]

Inflammatory cell infiltration beneath the surface or throughout the mucosa without loss of body glands.

* Loss of normal body glands with or without metaplasia

TPA patients later develop Addison's pernicious anemia (APA) (1 6, 13) The age distribution of TPA patients is nearly the same as that of APA patients the disease often begins later in life (10 18) TPA patients generally have atrophic gastritis (14) and gastric achlorhydria (3 10 12 14) However, disordered vitamin B_{12}-absorption measurable by the Schilling test is encountered in tapeworm carriers in proportion to the incidence of both atrophic gastritis (17) and achlorhydria (11 17) In the present series, the increase in the Schilling values caused by IF concentrate was correlated with the severity of the gastritis This observation seems to support the importance of the role of IF deficiency in the tapeworm carrier's B_{12}-absorption disturbance and the development of TPA

However it is known from in vitro experiments that the tapeworm is capable of releasing a greater proportion of gastric juice bound B_{12} than of hog IF-concentrate bound B_{12} (9) The hog IF induced increase in the Schilling values of tapeworm carriers could thus be explained on the basis of the species specifity of the B_{12}-releasing factor of tapeworm. But if this were the only consideration, what is the explanation of the correlation between the IF induced increase in the Schilling values and the atrophic changes of the gastric mucosa? Furthermore, if the established facts cited above are recalled the significance of IF deficiency in the etiology of TPA cannot be disregarded.

Summary

Gastric suction biopsy was taken from 28 carriers of fish tapeworm (*Diphyllobothrium latus*) and a Schilling test without and with IF was performed. Thirty per cent of the tapeworm carriers with normal gastric mucosa had an at least two-fold rise. The corresponding figure for those with atrophic gastritis was 85 per cent. The difference is significant ($P < 0.01$) The result supports the view that IF deficiency is concerned in the etiology of tapeworm pernicious anemia.

Acta Medica Scandinavica. Vol. 175 fasc. 6, 1964

Medical Department C (Head P From Hansen, M. D) Copenhagen County Hospital, Glostrup, and Medical Department B (Head Mogens Bjerneboe, M. D) Bispebjerg Hospital, Copenhagen, Denmark

Effect of Aldosterone on Orthostatic Circulatory Failure

By

J Ditzel, P H Hansen, E. Kemp and I F Lindbjerg

The great majority of Addisonian patients or bilaterally adrenalectomized individuals feel well on a substitution therapy consisting of cortisol and a supplement of salt. On this therapy however few patients show symptoms and signs indicating defective regulation of the blood pressure on changes in body position These extremely disagreeable symptoms often yield to further substitution therapy in the form of a suitable dosage of mineralocorticoid.

It is the object of the present report to illustrate the immediat effect of a small dose of d-aldosterone upon the orthostatic syndrome in an adrenalectomized patient. Moreover three case histories will be reported to indicate that aldosterone may be effective also in the treatment of other forms of orthostatic hypotension in patients with apparently normal adrenocortical function in whom other measures appear to be *ineffective*

Case reports

Case 1 A woman, aged 33, with history of appendectomy 20 and concussion at 21 Tw normal deliveries 9 and 6 years ago. During her latter pregnancy latent diabetes mellitus was diagnosed, and the baby weighed 3,950 g at birth. Admitted in October 1960 to the Gynaecological Department of the Copenhagen County Hospital, Glostrup with oligomenorrhoea. She had noticed that during the past years her ppearance had been changing. Her face had reddened and become more round. Because of a suspicion of Cushing s disease, the patient was transferred to medical department C. On admission here she was found to have a Cushingoid facies and neck, with large padding of fat over the shoulders, fairly pronounced hirsutism of the arms and legs and whiskers. The glucose-tolerance curve was of the diabetic type. B.P 155/110. 17 ketogenic steroids 14.9 mg/24 hours. ACTH stimulation test, using 20 I U in 500 ml physiological saline i. over 8 hours, gave a 17 ketogenic steroid excretion of 64.3 mg/24 hours. X-rays of sella turcica No abnormality Pneumoretroperitoneography No abnormality

During the subsequent period the patient complained even more of severe fatigue, hypersensitivity to noise, decreasing libido, and frontal headache. She was then readmitted in January 1961 This time she exhibited, moreover acne of the chest and striae distensae on the thighs B.P 160/110. 17-ketogenic steroids 19.0 mg/24 hours. Suppression test, using fluoxyprednisolone 4 mg twice daily for 5 days, showed only moderate suppression of the 17 ketogenic steroids to 11 1 mg/24 hours. Spon-

Submitted for publication November 4 1963.

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Case reports

Case 1 A woman, aged 33 with history of appendectomy at 20 and concussion at 21 Two normal deliveries 9 and 6 years ago. During her latter pregnancy latent diabetes mellitus was diagnosed, and the baby weighed 3,950 g at birth. Admitted in October 1960 to the Gynaecological Department of the Copenhagen County Hospital, Glostrup, with oligomenorrhoea. She had noticed that during the past years her appearance had been changing. Her face had reddened and become more round. Because of suspicion of Cushing' disease, the patient was transferred to medical department C. On admission here she was found to have Cushingoid facies and neck, with large padding of fat over the shoulders, fairly pronounced hirsutism of the arms and legs and whiskers. The glucose-tolerance curve was of the diabetic type. B.P 135/110. 17 ketogenic steroids 14.9 mg/24 hours. ACTH stimulation test, using 20 I.U in 500 ml physiological saline I. over 8 hours, gave 17 ketogenic steroid excretion of 64.3 mg/24 hours. X-rays of sella turcica No abnormality Pneumoretroperitoneography No abnormality

During the subsequent period the patient complained even more of severe fatigue, hypersensitivity to noise, decreasing libido, and frontal headache. She was then readmitted in January 1961 This time she exhibited, moreover acne of the chest and striae distensae on the thighs. B.P 160/110. 17-ketogenic steroids 19.0 mg/24 hours. Suppression test, using fluoxyprednisolone 4 mg twice daily for 5 days, showed only moderate suppression of the 17 ketogenic steroids to 11 1 mg/24 hours. Spon-

Submitted for publication November 4, 1963

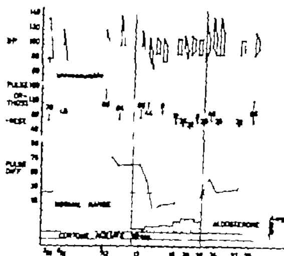


Fig 1 Blood pressure and pulse rate in the recumbent position and after 5 min. in the erect position as well as orthostatic pulse difference before, during, and after administration of aldosterone to an adrenalectomized woman (case 1). The orthostatic blood pressure is indicated by dots connected by vertical lines, the recumbent values being shown first. Concomitant pressure values on the same day are connected.

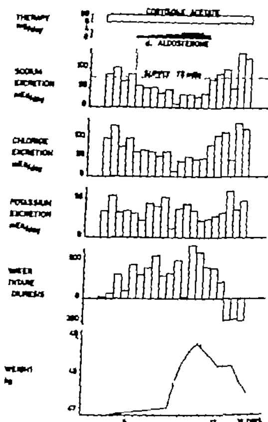


Fig 2 Urinary output of sodium, potassium, and chloride water balance and body weight before, during, and after aldosterone medication in case 1

taneous 17 ketosteroid excretion 9 1 mg/24 hours. Serum sodium 140 mEq/l Serum potassium 3.9 mEq/l. She was now amenorrhoeic. Owing to the progression of the symptoms and signs, she was transferred to a surgical department and submitted to bilateral adrenalectomy Both adrenals were hyperplastic. Microscopic diagnosis Hyperplasia of the adrenal glands (signed K. Schourup) After the operation she was put on a substitution therapy with 50 mg cortisone acetate and her appearance returned to normal.

Re-admitted in November 1961 Her main complaints were fatigue and palpitations, even upon minor exertion. On admission the B.P was on several occasions found to be 130/105 in the recumbent position and about 120/105 after standing for 5 minutes. At the same time, an abnormal increase in the pulse rate occurred an increase to about 70 per minute (normal value < 27 per minute (2)) In all orthostatic experiments she showed tendency to orthostatic collapse. It was now decided to treat the patient intramuscularly with d-aldosterone, in the form of Aldocorten® and during this experiment the patient was adjusted to a diet containing a fixed amount of sodium, 78 mEq daily

Fig 1 illustrates the effect of an increasing dose of d-aldosterone (0.5 mg—4 mg daily over 10 days) upon the orthostatic blood pressure and pulse Prior to aldosterone, the diastolic blood pressure in the erect position was frequently immeasurable, and there was an exaggerated difference between the pulse rate in the erect and recumbent position, and a tendency to collapse. On aldosterone medication the tendency to collapse disappeared. At the same time the orthostatic blood pressure and the orthostatic pulse difference were normalized Thus effect was obtained by 2 mg aldosterone daily over three days. After aldosterone was withdrawn there was a rapid relapse, with a tendency to collapse and an increase in the orthostatic pulse difference. Aldosterone medication resulted in a striking subjective as well as objective improvement which was maintained after changing to sublingual administration of 3 mg aldosterone daily

Fig. 2 shows the effect of aldosterone upon the urinary excretion of sodium, potassium and chloride as well as upon body weight and urinary output. During aldosterone medication total of about 403 mEq sodium was retained. At the same time, there was retention of fluid, as indicated by positive balance between intake and urinary output. She gained about 2 kg. After aldosterone was withdrawn the sodium output increased and the water balance became negative. It must be mentioned that despite increasing doses of aldosterone, from 0.5 mg to 4 mg, the retention of salt did not increase. A maximum retention appeared to be attained within 6 days on an aldosterone dose of 2 mg daily and the retention seemed to be less on 4 mg (possibly escape phenomenon") The urinary output of potassium showed no significant differences before and during the aldosterone medication. On the other hand, there was decrease in serum potassium from 4.8 mEq/l to 3.0 mEq/l. No oedema was observed.

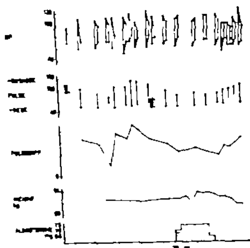


Fig. 3. Blood pressure and pulse rate in the recumbent position and after 5 min. in the erect position as well as orthostatic pulse difference and body weight before, during, and after aldosterone medication in case 2

Case summary

The effect of a 10-day aldosterone substitution therapy upon the orthostatic circulatory failure and upon the water and electrolyte balance in an adrenalectomized patient is illustrated. Aldosterone in doses of 2 mg resulted in a rapid improvement in the symptoms as well as signs of orthostatic circulatory failure.

In other forms of orthostatic hypotension too, such as orthostatic arterial anaemia and postural hypotension" aldosterone ppears to be effective as indicated by the following case histories.

Case 2. A 28-year-old woman of asthenic build was admitted to Medical Department C, Copenhagen County Hospital, Glostrup, for evaluation of headache. From childhood she had been suffering from psoriasis, but had otherwise had no major illnesses. Tw normal deliveries at "0 and 22 years of age

During the stay in hospital the patient complained of dizziness and malaise as soon as she rose from her bed. I orthostatic experiments of 5 minutes duration she responded by hypotension and pronounced increase in the pulse rat Several times she fainted before the experiment was completed.

Other findings Hb 130 g/l, serum potassium and sodium normal. ECG and X-ray of the heart and lungs N abnormality

The patient was then treated for 7 days with aldosterone in the form of Aldocorten® maximum dose of 0.5 mg i.m. 3 times daily

Fig 3 shows how the tendency to orthostatic collapse (coll.) present in the preliminary period, decreased during the treatment. At the same time, there was decrease in the orthostatic pulse difference. Her subjectiv complaints also disappeared during the treatment period, only to return after the drug was withdrawn

During the medication she gained 1.5 kg, but after withdrawal her weight returned to the previous level.

Case summary

A 28-year-old woman w th orthostatic arterial anaemia" and a tendency to collapse was treated w th aldosterone i.m. through a 7-day period. During this treat

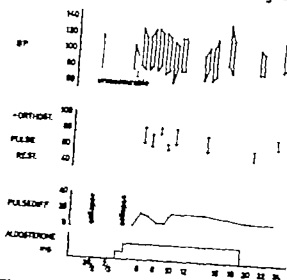


Fig 4 Blood pressure and pulse rate in the recumbent position and after 5 min. in the erect position as well as orthostatic pulse difference before, during, and after aldosterone medication in case 3.

ment her orthostatic circulatory failure and the attendant complaints disappeared.

Case 3. A 36-year-old dentist was admitted to Medical Department C, the Copenhagen County Hospital, Glostrup, for syncopal attacks. History of cholecystectomy at 30 and from the age of 32 treated with thyroid because of a low B M.R. At 35 he had been admitted for impotentia generandi the sperm count was 50 % below normal, but no hormonal dysfunction was found.

At the age of 36 the patient began to suffer from fainting spells of orthostatic type He would get dizzy while working and often had to sit down he experienced blackening before his eyes when he rose from a chair When these complaints had persisted for 3 months, the patient was admitted. The blood pressure in the recumbent position was 120/80 but an orthostatic test resulted in marked hypotension and fainting with, however no abnormal increase in the pulse rate (fig 4)

Other investigations, including ECG and X rays of the heart and lungs, revealed no abnormalities. The metabolic rate was normal.

First, he was treated with ephedrine hydrochloride tablets in doses of 25 mg every 6 hours for 48 hours, but this proved ineffective. Then aldosterone was started in tablet form, 1 mg 4 times daily After 36 hours medication the blood pressure remained relatively unchanged in a 5-minute orthostatic test, and in continued daily tests he went on showing a normal response (fig 4)

The treatment was continued on an out patient basis. The patient is now feeling well and has returned to work.

Case summary

A 36-year-old man suffering from "postural hypotension" with a tendency to collapse was treated for 16 days with oral aldosterone 4 mg daily During this medication his orthostatic complaints disappeared. On the other hand a sympathomimetic drug seemed ineffective.

Case 4 A 75-year-old woman with a history of operation for ovarian cyst in 1912, for cancer of the rectum in 1948, and for cancer of the right breast (mastectomy) in 1958. No signs of recurrence or metastases. Otherwise, the patient is in good health and surprisingly well-preserved, physically as well as mentally

Her present condition dated from the middle of December 1961 She was then admitted to Medical Department B, Bispebjerg Hospital, because of attacks of dizziness and fainting which would come on particularly when she rose. In addition, she was complaining of fatigue and weight loss. During the stay in hospital she was found to exhibit marked orthostatic hypotension, but all laboratory findings were normal, except for mild anaemia.

She was first treated with desoxycorticosterone and sodium chloride for a fortnight, but as this proved ineffective the medication was withdrawn.

Then aldosterone (fig 5) was instituted, 0 5 mg of Aldocorten ® intramuscularly and sodium chloride 10 g daily for 20 days. After a certain latent period the blood pressure in the erect position rose, and the patient reported subjective improvement. At the end of 20 days the medication was withdrawn, and placebo was given for 10 days, 1 ml physiological saline intramuscularly Strangely enough, the patient kept well, and the blood pressure remained constant. She was then discharged for out patient control. The stable condition persisted for about 3 weeks, but then the former symp-

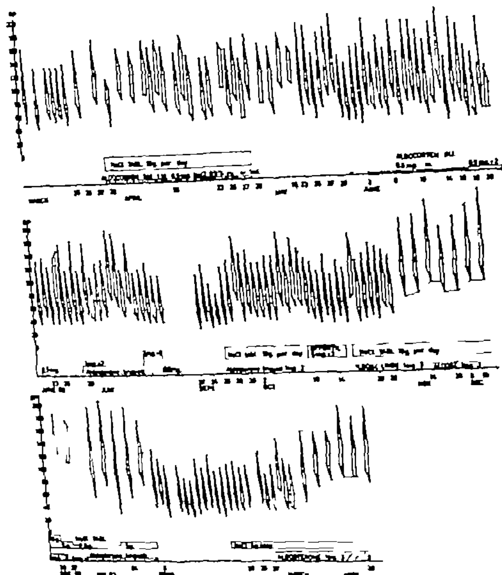


Fig. 5 Blood pressure in the recumbent position and after 5 min. in the erect position before, during, and after aldosterone sodium chloride and Efforil medication in case 4

toms of dizziness, tendency to collapse, and fatigue returned. Moreover the diastolic blood pressure in the erect position fell to very low values, on some day as low as 30 mm of mercury.

The patient was re-admitted and was again started on Aldocorten injections — this time without salt. Despite long treatment period, in which sublingual administration was tried as well, the patient did not respond satisfactorily. Not until the sublingual aldosterone medication of 1 mg 3 times daily was supplemented by 10 g sodium chloride daily did the blood pressure become stabilized on a higher

level and the general condition improve. After two weeks' treatment aldosterone was discontinued, and she was treated instead with Effortil® (dl 1 (3-hydroxyphenyl)-1 hydroxy-2-ethyl-aminoethan) tablets, 5 mg 3 times daily for one week (fig 5) During this treatment the orthostatic blood pressure fell, but returned to normal as soon as treatment with aldosterone + salt was resumed Now she was discharged on aldosterone and salt and attended as an out-patient once weekly She remained well (attending the out patient department on her own and managing her domestic duties) and the blood pressure was satisfactory

However she slowly developed hypertension, so that the dosage of aldosterone and salt had to be gradually reduced since it proved difficult to find the correct dosage, she was re-admitted. During this stay in hospital she was first taken off all medication, and as usual the blood pressure fell immediately until the medication was resumed, whereupon systolic hypertension re-appeared. During the stay in hospital her plasma steroids (cortisol, compound S, and corticosterone) were determined, and so was the urinary output of 17 ketogenic steroids and 17 ketosteroids. All values were normal. Serum electrolytes were also normal.

Case summary

A 75-year-old woman with "postural hypotension was treated over a long period with aldosterone and salt with a favourable effect upon the orthostatic circulatory failure. The treatment was complicated by hypertension which yielded after withdrawal of the medication

Discussion

The four reported case histories illustrate the effect of aldosterone upon three fairly well-defined types of orthostatic hypotension, viz. 1 the syncopal state in totally adrenalectomized patients, 2 Bjure and Laurell's orthostatic arterial anaemia" and 3 Bradbury and Eggleston's "postural hypotension (9)

The effect of aldosterone is perhaps most clearly apparent from case 1 who was indubitably suffering from aldosterone deficiency The maximum effect of aldosterone upon the orthostatic circulatory failure was evident as early as 3 days after the institution of intramuscular administration of 2 mg daily The pulse difference between the erect and recumbent position fell to normal (less than 27 per minute (2)) and the blood pressure was stabilized. At the same time, the characteristic effect of aldosterone upon the electrolyte balance was observed, viz. retention of sodium and water with a gain in body weight Like August et al. (1) we found that an increased dose of aldosterone, even in an adrenalectomized patient, did not give rise to an increased retention of sodium but that an "escape phenomenon appeared.

Ledingham et al. (5) Nelson and Cooper (6) and Worning and Rosenbeck Hansen (11) have shown that aldosterone is biologically active when administered by the oral or sublingual route to patients with Addison's disease. A sodium balance was achieved in all the cases by aldosterone 1—2 mg when administered sublingually and $^{1}/_{2}$—5 mg when administered orally (11) When aldosterone was given sublingually to our adrenalectomized patient the drug appeared effective in preventing symptoms and signs due to orthostatic circulatory failure.

On the basis of previous experience regarding the stabilizing effect of desoxycorticosterone upon the blood pressure of hypotensive subjects (4) it seemed natural to try aldosterone in similar cases. Searching the literature on this subject, we did not succeed in finding previous reports on the use of aldosterone apart from Schirger et al's (8) negative result in one case. Since our first three cases, reported above,

gave an apparently good response to this treatment, it seemed justified to publish this *preliminary* report.

Cases 2 and 3 showed considerable subjective as well as objective improvement on a short lasting treatment with 1.5 mg aldosterone intramuscularly and 4 mg aldosterone by mouth respectively. The blood pressure became stabilized, and the syncopal attacks disappeared, without any development of hypertension. Case 4 required a supplement of salt. Like the previously reported cases on long-term desoxycorticosterone, this patient developed hypertension. According to the literature, however, this complication of short term aldosterone therapy is said to be more uncommon than with desoxycorticosterone. Nevertheless this case shows the need for frequent checking of the blood pressure.

The reason for the effect of aldosterone in patients with orthostatic hypotension has not been clarified. That aldosterone is a factor in the regulation of the blood pressure is clearly apparent from the facts that a deficiency of aldosterone, as in Addison disease, results in a low blood pressure, and that an increased quantity of aldosterone, as in cases of primary hyperaldosteronism, leads to an elevated blood pressure. It is also indicated by the fact that the human adrenals secrete more aldosterone in the erect than in the recumbent position (5, 10). It is possible therefore that in patients suffering from orthostatic hypotension the adrenals are unable to react to changes in position by adequate changes in the secretion of aldosterone. Salt and water retention caused by administration of aldosterone gives rise to an increase in extracellular fluid and plasma volume, a factor which perhaps tends to increase the blood pressure. However the effect of aldosterone does not appear to act exclusively through this mechanism. Aldosterone also exerts a direct effect upon the small blood vessels, an effect which leads to arteriolar constriction. This arteriolar change is presumably caused by an increased sensitivity of the vessel wall to catecholamines or an oedema of the mural cells caused by sodium retention (7). Although as yet the clinical and experimental experience of aldosterone is meagre, it does indicate that the hormone plays an important role in homeostasis, and that the therapeutic possibilities of aldosterone ought to be tested beyond simple substitution therapy in clinically manifest adrenocortical insufficiency.

Summary

Four cases of orthostatic hypotension were apparently successfully treated with aldosterone, intramuscularly and orally. In one case, which required long-term medication, a supplement of salt was needed. This patient developed transient hypertension. The four cases represent three different types of hypotension. The cause of the effect of aldosterone is discussed.

Acknowledgement

The authors wish to thank Dr. J. Lorenzen, CIBA Limited, Copenhagen, for supplying the aldosterone used in this study.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Further Studies on Glucose Metabolism in Experimental Potassium Depletion

Effect of Insulin, Glucagon, and Muscular Exercise

By

UFFE SAGILD and VAGN ANDERSEN

In a previous study (8) we found in experimental potassium depletion in humans a considerable reduction in the rate of glucose disappearance from the blood following intravenous glucose loading and a comparable reduction in the mean cellular uptake of glucose. Insulin responsiveness appeared to be unaffected by potassium depletion but insulin was given 60 minutes after the glucose injection consequently the blood sugar was not in a steady state at the time of insulin administration.

In this study the effect of potassium depletion upon insulin responsiveness has been re-evaluated, insulin being given in the fasting state. Furthermore, the effects of glucagon administration upon hepatic glycogenolysis and of heavy work upon muscular glycolysis were studied before and after potassium depletion.

Material and methods

Four healthy males, age range 22–37 years, weight range 80–92 kg, were submitted to the tests. All continued their habitual daily activities during the study.

Submitted for publication November 11, 1963.

Test procedures

The following three tests were performed before and after period of potassium depletion.

Insulin test. 8 units of hyperglycemic-factor free insulin were given intravenously. Samples of arterialized blood from the ear-lobe were obtained at three to five minutes intervals for 60 min. and analyzed for glucose. Every 30 min. a venous blood sample for serum potassium analysis was collected.

Glucagon test. In accordance with van Itallie and Bentley (5) epinephrine was given prior to glucagon administration. 0.4 mg epinephrine was injected subcutaneously and 10 min. later 2.0 mg glucagon (Novo Medical Factory) intramuscularly. This modification of the test had in preliminary experiments been shown to give reproducible results. Arterialized and venous blood samples were obtained before the injection of epinephrine and every 15 min. during the first hour of the test, every 30 min. during the next two hours.

Exercise test. The subjects performed work at about 2,000 kg/min. on bicycle ergometer. The work load was chosen on previous experiments according to individual strength. The same performance was required before and after potassium depletion. One working period of 1 min. and 3 of 30 sec. were separated by rest periods of 30 sec. Before the test, in the intervals, and 3, 6, and 9 min. after exercise,

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changed. The electrocardiographic tracings revealed the development of flattening of the T wave in one subject, but in the others they remained unchanged. The depletion caused no subjective symptoms except some degree of muscular fatigue. One subject, however developed vaso-vagal attacks of moderate intensity during the insulin and exercise tests after depletion.

Insulin test

The blood-sugar concentration following insulin administration declined exponentially with time during the first 25 minutes of the test the rate of decline of the blood sugar during this period may be expressed as K_1 = the percentage fall in blood-sugar per minute. The K values of arterialized blood before potassium depletion varied between 4 8 and 5 5 This is in accordance with the values reported by Franckson (3) who used venous blood.

After depletion, K_1 remained unchanged in one subject, showed a small and insignificant reduction in two cases, while in the fourth the results were discarded because the subject developed vaso-vagal attack immediately following insulin injection.

The lowest blood-sugar concentration was attained 24—29 minutes after the insulin injection being on an average 37 mg% before and 40 mg% after potassium depletion The blood sugar reached approximately normal values within 60 minutes in all tests.

Fig 1 shows that insulin injection provoked a fall in serum potassium concentration this was of the same magnitude before and after potassium depletion. The restoration of serum potassium, however seemed to be more rapid when the potassium concentration was low

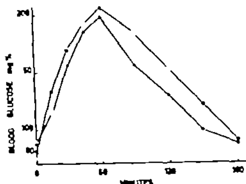


Fig. 2. Blood-glucose concentration following administration of epinephrine and glucagon.
○—○ before depletion, +—+ after depletion.

Glucagon test

The administration of epinephrine and glucagon elicited in all subjects a hyperglycemic response which in no instance was significantly altered by potassium depletion (fig 2) Maximal blood-sugar concentrations were attained at 45—60 minutes the rise was in three subjects about 100 mg% in the fourth 80 mg%. During the next two hours the blood-sugar again decreased the rate of fall was not influenced by potassium depletion. At the end of three hours, the initial blood-sugar concentration was attained in most cases.

Exercise test

The acute heavy work produced in all subjects a considerable degree of metabolic acidosis (fig 3) During the exercise period the pH of arterialized blood decreased on the average 0 13 and base excess fell from + 2.0 to — 10.3 mEq/l. According to Mellemgaard and Astrup (6) this corresponds to an extracellular acid accumulation of about 320 mEq in these subjects. Nine minutes after exercise a significant acidosis was still present.

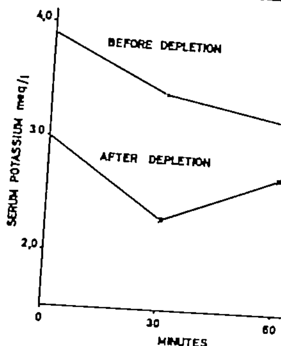


Fig 1 Average values of serum-potassium concentration during the Insulin test.

arterialized blood was obtained. By the method of Astrup et al. (1) actual and equilibrated pH of the blood were measured, and from these data P_{CO_2} and base excess were calculated.

The tests were done on three consecutive days after an overnight fast.

Balance procedure

Potassium depletion was induced over a period of 7 days by the administration of the cation-exchange resin Amberlite IR 120 in the ammonia form. Each subject received 75 g per day in divided doses with each meal. During the test days following the depletion period the dose of resin given was reduced to 30 g a day in an attempt to sustain but not augment the degree of potassium depletion

The diet provided during the study varied somewhat between individuals according to their need but was held rigidly constant for the same person throughout. It provided approximately 60 mEq of potassium, 25 mEq of sodium, 75 g of protein, and 2 600 cal. per day according to appropriate tables. Additional glucose and water were taken ad lib.

The excretion of potassium, sodium, and nitrogen during the depletion period was assessed by analyses on feces and urine, the feces corresponding to the experimental period being determined by using the resin itself as a feces marker In addition, the urinary excretion of acid, glucose, and 17 keto-steroids was studied. Daily determinations of serum potassium, sodium, chloride, and standard bicarbonate were performed.

The analyses for glucose were made in duplicate by the Hagedorn-Jensen method (4) Some of the analyses were performed at the Central Laboratory of the University Hospital (Head P Astrup, M. D) The measurements of blood hydrogen ion concentration were performed by the micromethod of Astrup et al. (1) The remaining analyses were performed according to standard laboratory methods.

Results

Balance procedure

The depletion induced a loss of 220—420 mEq of potassium in the four subjects. This corresponds to 5—10 per cent of the total body potassium pool (7) Corrections for the nitrogen balance, which was slightly negative in three instances and positive in the fourth and for the decrease in serum potassium (see below) showed that the intracellular potassium loss was of the same order of magnitude (8)

Serum potassium decreased by 0.9—1 5 mEq/l from the initial normal levels, the lowest observed value being 2 5 mEq/l. The levels of serum sodium, chloride, and standard bicarbonate remained unchanged.

With the exception of an unexplained negative sodium balance in one instance (275 mEq) the results of the depletion procedure were in fairly close agreement with those obtained in our previous study using the same method

The fasting blood sugar (arterialized blood) increased by 7—14 mg% during the depletion period None of the urine specimens contained glucose. The urinary excretion of 17 keto-steroids remained un

vas. In the present series of experiments the hyperglycemic response to glucagon was not affected significantly by potassium depletion. This suggests that hepatic output is not materially altered in this condition.

Therefore a decreased peripheral uptake of glucose seems to be the most likely explanation of the slower glucose disappearance after potassium depletion. If it is assumed that sensitivity to exogenously administered insulin (which was found to be unaffected by potassium depletion) reflects responsiveness to endogenously produced hormone, it may be inferred that potassium deprivation in some way affects the availability of endogenously produced hormone under the conditions of glucose loading.

The exercise test was devised in order to determine whether the muscular fatigue which is a common symptom in potassium depletion could be related to quantitative changes in the acid released from muscles during standardized heavy work. Under the conditions of the study a considerable degree of acidosis was produced before as well as after potassium depletion, but no quantitative differences were observed.

Summary

Potassium depletion was induced in four normal subjects by oral administration of a cation exchange resin. The resulting potassium loss was estimated at 220—420 mEq.

Before and after potassium depletion, the insulin-induced hypoglycemia, the glucagon-induced hyperglycemia, and the acidosis resulting from heavy muscular work were studied. None of these parameters were affected by a potassium depletion of this magnitude.

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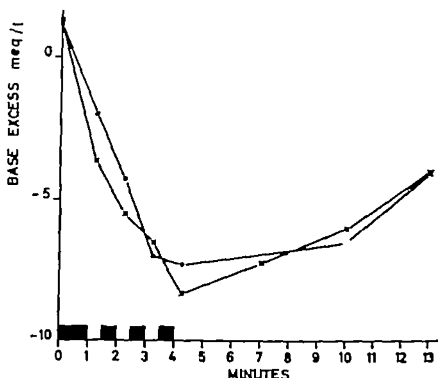


Fig. 3 Changes in base excess resulting from muscular work before and after potassium depletion. ●—● before depletion, ×—× after depletion, ■■ exercise periods.

The degree of acid accumulation was not affected by potassium depletion in any of the subjects.

Discussion

In our previous work (8) it was shown that following potassium depletion the rate of disappearance of glucose injected into the blood stream (K_G) was significantly reduced. In order to determine whether this was due to a diminished insulin responsiveness, insulin was administered 60 minutes after the glucose loading and the rate of glucose disappearance from the blood (K_I) determined. No indication of a decreased insulin sensitivity in potassium depletion was found. However the blood sugar was not in a steady state at the time of the insulin administration and the initial glucose concentration of the blood was not identical before and after potassium depletion, being higher in the latter situation.

Although it has been demonstrated by Bishop and Marks (2) that differences in initial glucose concentration do not affect K_I in duplicate experiments, it was felt that a re-evaluation of the influence of potassium deprivation upon K_I was indicated insulin being given in the fasting state

In the present study it was confirmed that depletion of total body potassium by approximately 5—10 per cent does not affect K_I Insulin sensitivity thus appears to remain unaltered

The reduced rate of glucose disappearance following potassium deprivation might conceivably result from an increase in hepatic glucose output. Glucagon the hyperglycemic factor of the pancreas, has been shown to produce hyperglycemia by stimulating selectively hepatic glycogenol

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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The Effect of Sodium Salicylate on the Uptake of ^{131}I labelled 1 triiodothyronine by Human Erythrocytes

By

H. Hvid Hansen and Erik F Mogensen

During recent years, the method described by Hamolsky and Freedberg (4) for determination of the in vitro uptake of I^{131}-labelled 1 triiodothyronine in erythrocytes has been widely used in clinical studies of thyroid function. In their original paper Hamolsky et al. stated that the results of the test are affected by a number of factors which are independent of function of the thyroid gland, such as oestrogen, dicoumarol and salicylic acid.

The present paper is a report of experiments in which the influence of salicylic acid on the test was studied both in vivo and in vitro

Methods and material

In-vivo method

The patients were given sodium salicylate 1 g three times daily for 4 days. The measurements were performed as described below under the in vitro method, but without the addition of salicylic acid.

Blood from patients free from any suspicion of thyroid disorders was used in the experiments. The PBI showed normal results in all patients examined.

The serum concentrations of salicylic acid were estimated by the method of Routh et al. (7)

In-vitro method

A volume of about 20 ml venous blood drawn from non-fasting donors and patients was stabilised with 0.25 ml potassium oxalate and centrifuged at 2,000 r.p.m. for 10 min. The plasma was pipetted off and the erythrocytes were washed once with physiological saline. Duplicate samples of 3 ml plasma and 3 ml washed erythrocytes were transferred to conical flasks.

In order to obtain the various concentrations of salicylic acid given in table II approximately calculated amounts of a 25 % w/v salicylic acid solution were added to only one of the flasks.

Both flasks were gently shaken and then incubated at 37° C for 45 min. The shaking was repeated every 15 min.

^{131}I-labelled 1-triiodothyronine was then added to both samples in amounts such that the concentrations did not exceed 0.1 μg/ml.

The samples were again incubated at 37° C for 120 min., with gentle shaking every 15 min. During both periods of incubation the flasks

Submitted for publication November 11 1963.

Table I. The effect of salicylate treatment (mg/100 ml serum) on the uptake of T (%)

Patient	Days	1	2	3	4	5	6	7
K. L.	T	5.5	7.6	—	9.3	—		
	Sal.	0	5	—	14	—		
B. S.	T	7.5	—	8.6	6.7	11.5	7.2	—
	Sal.	0	—	3	0	6	0	
S. H.	T	6.6	8.2	—	5.8	—	7.7	—
	Sal.	0	18	—	29	—	22	—
I. F.	T	5.4	—	8.0	—	—	8.9	8.6
	Sal.	0	—	26	—	—	31	22
R. B.	T	6.7	—	7.9	—	—	7.1	7.0
	Sal.	0	—	18	—	—	5	0
K. L.	T	6.1	—	9.1	—	9.1	8.7	—
	Sal	0	—	8	—	30	21	—
G. H.	T	6.1	—	8.1	—	9.1	9.8	—
	Sal	0	—	14	—	29	30	—
T. R.	T	5.7	—	6.5	—	6.5	—	5.0
	Sal.	0	—	5	—	0	—	0
V. M.	T	6.1	—	9.4	—	10.5	—	8.4
	Sal.	0	—	3	—	12	—	13

study the conditions within a rather narrow range of concentrations.

In order to study the mode of action of salicylic acid, experiments were performed in which erythrocytes were washed six times and then suspended in physiological saline instead of plasma. Table III shows that under these conditions the erythrocyte uptake of T is not increased by salicylate, i.e. salicylic acid has no influence on the surface of the erythrocytes.

Accordingly it must be concluded that salicylate exerts its influence on the binding of T_3, to the plasma proteins.

Table II. Effect of salicylic acid on the in vitro uptake of ^{131}I-labelled T by human erythrocytes (normal range 5.5—10.7%)

No. of exp	Concentr. of salicylate (mg/100 ml)	Mean uptake of T ( )
[illegible]	0	8.1±1.3
[illegible]	21	11.6±1.3
40	41	13.9±2.3
34	83	16.0±2.8
17	123	19.6±2.4
19	208	24.3±4.0
70	418	25.0±5.3

Table III. Effect of salicylic acid on the in vitro uptake of I-labelled T by human erythrocytes suspended in solution of sodium chloride concentration of salicylate 100 mg/100 ml

T uptake

Without salicylate (%)	With salicylate ( )
81.0	79.6
82.7	82.7
92.0	93.3
91.3	86.6
97.3	92.7
91.0	90.1

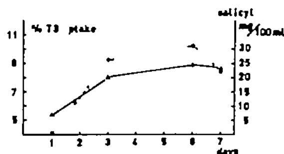


Fig. 1 T uptake and salicylate concentration in experiment in patient J F Δ—Δ T uptake, o—o salicylate.

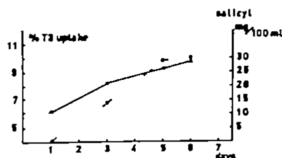


Fig 2 T uptake and salicylat concentration in experiment in patient C. H Δ—Δ T uptake, o—o salicylate.

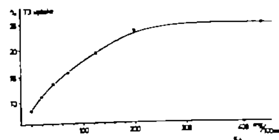


Fig. 3. The in vitro T uptake with increasing salicylate concentration.

were plugged with rubber stoppers. After the second incubation 2 × 2 ml blood were rapidly pipetted off from each flask into four centrifuge tubes.

The blood cells in the first tube from each sample were washed three times with 6 ml physiological saline. After the last washing and removal of the supernatant the activity of the cells was counted in a scintillation counter ("washed erythrocytes")

Similarly the activity of the blood cells in the second tube from each sample was counted in a scintillation counter ("unwashed erythrocytes")

The results were calculated as follows

$$\frac{\text{Activity in washed erythrocytes} \times 100}{\text{Activity in unwashed erythrocytes}}$$

The results are thus given for 50 % haematocrit and, accordingly further correction is unnecessary

Donor blood from the Blood Bank, Aarhus Kommunehospital, was used in the experiments.

Results

In vivo

As appears from table I administration of salicylate results in an increase in the T uptake in the red blood cells of the persons given salicylate. Representative curves are shown in figs. 1 and 2.

In vitro

The influence of the addition of salicylic acid in vitro was studied and an increase of the T_3 uptake was found. The increase in vivo was compared with the effect of addition of salicylic acid to a concentration of the same magnitude as that obtained in vivo. The results are given in table II and fig 3 It is seen that the addition of salicylic acid appreciably increases the T uptake by the erythrocytes. Hence the effect of salicylic acid is not exerted indirectly through metabolic processes, but directly on the T_3 binding components. It is also seen that the T_3 uptake does not increase proportionally with the concentration of salicylic acid but that the curve is flattened out at concentrations exceeding about 200 mg%.

The increase obtained in vivo seems to be identical with that observed in vitro at the same salicylic acid concentration. However in vivo it is only possible to

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Further Studies on Liver Metabolism in Subjects with Gallbladder Cholesterol Stones

Liver Content of Acetoacetate and Cholesterol

By

L. Villa, G. Idéo, A. Agostoni and N. Dioguardi

Fatty acid oxidation by the hepatic tissue of patients affected by gallbladder cholesterol stones, without signs of hepatic involvement, was studied in a previous paper (21). In these patients the oxidation of fatty acids (such as linoleic and octanoic acids) studied by Lehninger's method (9) occurs normally through the Krebs cycle, while the metabolic route leading to the synthesis of acetoacetate (AcAc) is completely abolished. These studies were carried out on whole homogenates. The results were later confirmed. Since, as Lehninger has shown, fatty acid oxidation occurs at the mitochondrial level studies on isolated mitochondria were also attempted. However the quantity of the human bioptic tissue was not sufficient to perform this type of study on mitochondria. On the other hand, the use of total homogenates has in retrospect conferred particular validity on these data, as recent research has revealed that some of the steps of fatty acid oxidation occur in microsomal structures (13).

To obtain some direct confirmation of the previous results, the AcAc content of the liver in cases of gallbladder cholesterol stones was determined. Moreover the hepatic cholesterol content was also assayed since it has recently been reported that one of the most important steps leading to AcAc synthesis has a common stage with cholesterol synthesis (the hydroxy-methyl-glutaryl-coenzyme A [HMGCoA]) so that an equilibrium between cholesterol and AcAc synthesis has been suggested (2). The ratio between the hepatic AcAc and cholesterol content was studied by carrying out a series of experiments on the hepatic tissue of rats fed with unbalanced diets able to influence fat metabolism.

Material and methods

Two groups of patients were studied. The first was formed of patients affected by non-icterogenic cholesterolic cholelithiasis. Subjects operated for gastric ulcers or neoplasm without hepatic metastases were used as the control group.

Submitted for publication November 12, 1963.

Finally it was checked whether the concentrations of salicylate had any influence on the pH of the blood. But even the highest concentrations observed did not bring about any changes in pH

Discussion

Triiodothyronine is found in the blood partly in a free form and partly bound to the plasma proteins and erythrocytes and the equilibrium of the system must be assumed to follow the law of mass action.

It was previously shown that salicylate affects the transport of thyroxine. Thus Austen et al (1) showed that salicylate accelerates the peripheral liberation of thyroxine in vivo It has also been demonstrated that salicylate increases the rate at which thyroxine dialyses through a membrane in in-vitro experiments (2) This was confirmed by Ingbar (5) who found that the influence on l thyroxine was more pronounced than that on d thyroxine.

Our experiments have shown that it is the binding of T_3 to plasma protein which is affected while the binding of T to the erythrocytes remains unaffected by salicylate. This is only partially in agreement with the statement by Golden and Osorio (3) who expressed the opinion that salicylates exert an influence on all triiodothyronine-bearing plasma proteins. Their statement seems not to agree with our experiments, which show that salicylate increases the T uptake only up to a certain limit. The simplest explanation of this is that salicylate affects only one or two of the triiodothyronine bearing plasma proteins and possibly blocks only certain sites in a single area of the protein However the actual mechanisms of binding are still unknown so that nothing definite can be stated

Furthermore it should be mentioned that the influence of salicylate on the T_3 uptake may be secondary to its action on thyroxine. Salicylate brings about liberation of thyroxine from the proteins, and the liberated thyroxine may then compete with T_3 for the remaining sites which are common to both hormones. As thyroxine has a greater affinity for the proteins than triiodothyronine, the portion of T which is not bound to the plasma proteins may thus be bound to the erythrocytes.

Summary

Salicylate has been found to increase the ^{131}I triiodothyronine uptake of the red cells but only to a certain degree. It is shown that salicylate affects the binding of T_3 to the plasma proteins and not the binding of T_3 to the red cells. These findings have been discussed in relation to the results of other investigators.

Acknowledgement

The authors are indebted to Dr Kiemeyer, Aarhus Kommunehospital for obtaining blood from the blood-donors. Our thanks are due to Dr C. E. Andersen, Centrallaboratoriet, Odense Amts og Bys Sygehus for the salicylate determination in serum from the in-vivo experiment.

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On the contrary the cholesterol of this group of patients had greatly increased (table II)

The results obtained from the livers of fasting rats indicate an increase in the AcAc content which becomes increasingly obvious when fasting is prolonged for 48 hours (table III) and a fall in the cholesterol content (table IV)

Table V shows the variations in the hepatic AcAc content and the cholesterol content for rats placed on Handler s diet. Examination of this table shows that the AcAc falls notably during the first 10 days of the diet. The AcAc returns to values higher than the starting level after 10—15 days. The cholesterol content on the contrary follows an opposite course.

Table IV Cholesterol content of the liver of rats kept on free diet (the data are expressed in mg of cholesterol/g of fresh liver ± SE)

Fed	Fasting	
	24 hours	48 hours
2.70	_80	2.50
3.00	2.90	2.10
3.20		2.20
3.50		2.00
3.00		2.00
2.50		
3.60		
3.50		
3.40		
Mean 3.15±0.12		Mean 2.10±0.10 ($p<0.05$)

Table V Acetoacetate and cholesterol content of the liver of rats kept on Handler' diet (the data are expressed in μg of acetoacetate/g of fresh liver and in mg of cholesterol/g of fresh liver)

Day on Handler diet	Acetoacetate	Cholesterol
0	10 63	3.15
2	8.50	—
5	2.00	7 10
5	1.50	10.50
8	2.70	—
8	1 70	—
8	1.00	—
8	1.90	—
10	3 00	21.00
10	2.40	24.00
10	6 10	—
15	17.00	12.00
15	17.50	14.00
15	12.30	—
20	17 10	15.60
21	16.50	16.20
22	17.00	—

Discussion

The results of these studies confirm the existence of a metabolic defect in patients affected with gallbladder cholesterol stones. In fact, the absence of fatty acid oxidation along the AcAc route, reported in previous work (21) is now confirmed by the reduced hepatic AcAc content in such subjects. It has also been possible to show an increased cholesterol content in the liver These data confirm the existence of a competetive mechanism between cholesterol and ketogenesis as has been suggested by various authors (2, 20) It is known that the synthesis of AcAc is brought about by two main mechanisms, one originating from acetoacetyl-coenzyme A (AcAcCoA) through a deacylase activity (17 18) and the other more important, from acetyl CoA through the HMGCoA cycle (3 11 20)

The scheme in fig 1 depicts the competition between keto- and cholesterol genesis that occurs at the HMGCoA level. A state of ketosis may occur either through increased desmolase activity or else through lowered TPN reductase activity (20) In addition to an increase of AcAc, an increase of HMGCoA was observed in

Table I Acetoacetate content of human liver (the data are expressed in μg of acetoacetate/g of fresh hepatic tissue ± SE)

Non-cholelithiasic subjects	Subjects with cholesterolic cholelithiasis
3.60	1.50
7.20	1.80
3.50	1.30
4.50	1.00
4.00	1.70
4.55	2.30
	2.90
Mean 4.56 ± 0.68	Mean 1.78 ± 0.24 ($p<0.01$)

Table II Cholesterol content of human liver (the data are expressed in mg of cholesterol/g of fresh liver tissue, ± SE)

Non-cholelithiasic subjects	Subjects with cholesterolic cholelithiasis
4.30	4.60
4.10	5.00
3.60	5.30
4.40	7.60
4.80	5.00
	5.40
Mean 4.24 ± 0.15	Mean 5.48 ± 0.43 ($p<0.05$)

Hepatic parenchyma, in amounts of approximately 500–1 000 mg, was obtained by biopsy during laparatomies performed in the surgical ward, on subjects kept on the same diet before operation and undergoing standardized anaesthesia and pre-anaesthesia. The biopsy samples were immediately placed in a refrigerator and utilized according to the technique described below

Experiments on rats have been carried out with the Sprague-Dawley strain the weight of the animals was approximately 200–250 g The animals were kept on a standard balanced diet.

Table III Acetoacetate content of liver of rats kept on a normal diet (the data are expressed in μg of acetoacetate/g of fresh liver tissue, ± SE)

Fed	Fasting	
	24 hours	48 hours
10.80	25.00	40.00
11.00	25.90	36.00
10.70	25.90	48.00
10.70	18.40	37.00
10.50	23.60	40.10
10.90		
12.10		
9.40		
12.00		
9.00		
12.10		
8.40		
Mean 10.63±0.34	Mean 23.76±1.34 ($p<0.001$)	Mean 40.22±2.33 ($p<0.001$)

Some groups of rats were kept fasting for 24 or 48 hours. Water was freely available.

Another group of animals was fed on a fatty diet according to Handler (7) The animals were sacrificed by decapitation and the livers utilized immediately

Acetoacetate The hepatic tissue was homogenized in distilled water in the proportions of 1 4 using Elvehjem-Potter apparatus. The proteins were precipitated with trichloroacetic acid (25 %) added to 3 %. The precipitate was removed by centrifuging and the AcAc assayed on the supernatant liquid, using Walker s method (19)

Cholesterol. The cholesterol was assayed according to the Abell Lewy Brodie method, after extraction according to the method of Sperry and Webb (1)

Results

Table I gives the results for the assay of AcAc in the liver of subjects affected with anicteric cholesterolic cholelithiasis and of control subjects. The results show a marked reduction of the AcAc contents in patients with calculi.

On the contrary the cholesterol of this group of patients had greatly increased (table II)

The results obtained from the livers of fasting rats indicate an increase in the AcAc content which becomes increasingly obvious when fasting is prolonged for 48 hours (table III) and a fall in the cholesterol content (table IV)

Table V shows the variations in the hepatic AcAc content and the cholesterol content for rats placed on Handler's diet. Examination of this table shows that the AcAc falls notably during the first 10 days of the diet. The AcAc returns to values higher than the starting level after 10—15 days. The cholesterol content on the contrary follows an opposite course.

Table IV Cholesterol content of the liver of rats kept on free diet (the data are expressed in mg of cholesterol/g of fresh liver ± SE)

Fed	Fasting	
	24 hours	48 hours
2.70	[illegible]	2.50
3.00	2.90	2.10
3.20		2.20
3 50		2.00
3 00		2.00
2.50		
3.60		
3.50		
3.40		
Mean 3.15±0.12		Mean 2.10±0.10 ($p < 0.05$)

Table V Acetoacetate and cholesterol content of the liver of rats kept on Handler' diet (the data are expressed in µg of acetoacetate/g of fresh liver and in mg of cholesterol/g of fresh liver)

Days on Handler diet	Acetoacetate	Cholesterol
0	10.63	3.15
2	8.50	—
5	2 00	7 10
5	1.50	10.50
8	2.70	—
8	1 70	—
8	1.00	—
8	1.90	—
10	3.00	21.00
10	2.40	24.00
10	6 10	—
15	17.00	12.00
15	17 50	14.00
15	12.30	—
20	17 10	15.60
22	16 50	16.20
22	17.00	—

Discussion

The results of these studies confirm the existence of metabolic defect in patients affected with gallbladder cholesterol stones. In fact, the absence of fatty acid oxidation along the AcAc route, reported in a previous work (21) is now confirmed by the reduced hepatic AcAc content in such subjects It has also been possible to show an increased cholesterol content in the liver These data confirm the existence of a competetive mechanism between cholesterol and ketogenesis, as has been suggested by various authors (2, 20) It is known that the synthesis of AcAc is brought about by two main mechanisms, one originating from acetoacetyl-coenzyme A (AcAcCoA) through a deacylase activity (17 18) and the other more important, from acetyl CoA through the HMGCoA cycle (5 11 20)

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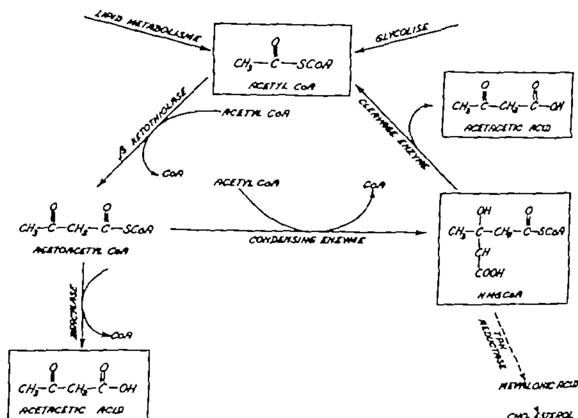


Fig. 1

diabetes and in fasting conditions, but as Bucher et al (3) and Wieland et al (20) have demonstrated there is an increase of desmolase activity in the first case, while in fasting conditions desmolase activity remains unchanged and the TPN reductase activity is blocked

Thus in fasting conditions, the cholesterol falls (4 16 22) Our experimental data confirm exactly the increased AcAc level and the fall of the cholesterol in the liver of fasting rats.

An inverse behaviour of these compounds under the stimulus of a fat rich diet (Handler diet) is demonstrated However the excessive accumulation of cholesterol may regress after a prolonged period on this diet

It can be concluded that AcAc synthesis is blocked and cholesterol increased in the liver of subjects affected by gall bladder cholesterol stones. In the present state of our studies, although we still cannot definitely state if the reduced AcAc formation is due to a fall in desmolase activity or to a primary increase in TPN reductase activity we can suggest the hypothesis that the metabolic disturbance occurs at the demolase level resulting in lack of fatty acid oxidation through the AcAc route Considering the frequent familial association of cholesterolic cholelithiasis and the ratio between cholelithiasis and blood groups (6 8, 10, 12 14 15) one may speculate on the genetic origin of this metabolic error

Summary

The AcAc and cholesterol content of the hepatic tissue of patients affected by gallbladder cholesterol stones has been

determined. While the AcAc was found to be greatly reduced, the cholesterol was increased.

The quantity of AcAc and cholesterol in the liver of rats kept in fasting conditions or on fat diet for several days was also assayed. The results reveal a distinct increase of AcAc and a reduction of cholesterol in the liver of fasting rats, while the hepatic AcAc content of rats on Handler's diet fell during the first ten days of the diet and the cholesterol showed a marked increase.

These studies point to a competitive cholesterol- and keto-genesis mechanism in the liver. This equilibrium would be modified in favour of cholesterol synthesis in patients with cholesterol stones. It is possible that this metabolic error plays a role during the formation of the cholesterol stones.

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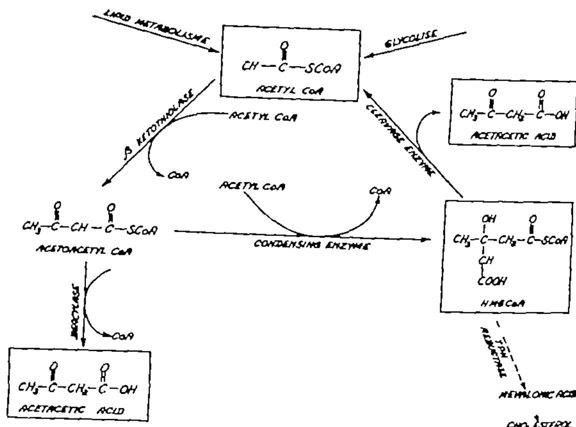


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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Fatal Right Ventricular Perforation During Cardiac Catheterization

By

Jørgen B. Dalgaard

When, in 1929 Forssmann first introduced a catheter into the right side of his heart, this was considered heroic. Today right cardiac catheterization (c.c.) is a standard procedure in cardiological laboratories, often supplemented by angiocardiography and sometimes by left cardiac catheterization

The procedures are mostly performed in patients with severe congenital or acquired heart lesions for evaluation of the prognosis or operability but the indications are sometimes extended to include a pathophysiological evaluation for insurance or other purposes. In the author's opinion, this is justified only if the procedure can be considered safe in such cases. But is it so?

An authoritative survey of the complications occurring in 5,691 right cardiac catheterizations was given by a special committee of the American Heart Association (4) Arrhythmia and right bundle-branch block pyrogenic reactions, and local irritation at the site of introduction were not unusual. Minor pulmonary infarction sometimes followed prolonged recording of the pulmonary capillary pressure. The syncopal syndrome with dilatation of pupils, pallor bradycardia and hypotension proved fatal in isolated cases (four deaths in the series) Mechanical complications included loops and angulations of the catheter air embolism and direct trauma to the endocardium (exceedingly rare in man")

The committee stated that angiocardiography implies a certain risk of syncope and mentioned several deaths during, or immediately following injection of contrast (5) Concerning catheterization via the arterial route, myocardial injury torn aortic valves, unintended coronary catheterization, and cerebral embolism were mentioned by the committee, and extreme caution advocated.

At the second European congress of cardiology in Stockholm 1956 Scebat et al. (13) reported the French experiences from 2,300 right heart catheterizations. Twelve cases were fatal, three due to direct trauma to the heart by the catheter At the same meeting the Italian authors Levi et al. (10) reported three deaths in 1150 right cardiac catheterizations and five in 1620 cases of angiocardiography

Submitted for publication November 13 1963.



Fig 1 Roentgenogram displaying the catheter partly in the pericardial sac.

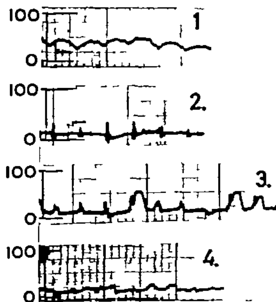


Fig 2. Pressure curves recorded at four positions during withdrawal of the catheter from a suspected position in the right pulmonary artery to the right atrium. Recording no. 4 may be from the right atrium, but recordings no. 1—3 are without doubt from the pericardial sac, nos. 2 and 3 showing irregular pressure oscillations at low mean pressure level.

According to Swedish reports (1) the rate of fatal complications of right c c. was 5/5 859 in angiocardiography it was 9/2 451 and in thoracic aortography 5/340 Fatalities occurred mainly in extremely poor patients. No deaths were due to cardiac tamponade. The survey of Wood and Swan (16) from the Mayo Clinic reported four deaths (none due to tamponade) in 1 710 cases of right c.c.

Caldini et al. (3) reviewed 31 deaths during or shortly after right c.c. and ascribed 16 to arrhythmia, 7 to gross anatomical lesions, while 8 cases were questionable.

The risk of cardiac tamponade

Right heart catheterization in isolated cases has been complicated by perforation of the pericardial sac.

McMichael and Mounsey (12) reported a non-fatal perforation, probably through a cardiac vein In the case of Stern et al (15) injected diodrast spread over the surface of the heart, without immediate untoward sequelae. Goodwin (7) reported a fatal case with haemopericardium and a small endomyocardial lesion following right cardiac catheterization in a 44-year-old patient with severe aortic stenosis.

In Escher et al s unique case (6) 50 ml of urokon was injected during right c.c. in a 24-year-old woman. The radiographs revealed contrast in the myocardium and pericardial cavity Cardiac tamponade supervened and pericardial incision was performed. The course was uneventful

Lurie and Grajo (11) reported four accidental cardiac punctures during right c.c in critically ill children Two perforations of the right auricle caused fatal tamponade. Two occurred in the right ventricle one was non-symptomatic, the other indirectly fatal.

Methods for *left heart catheterization* involve a much higher risk of complicating haemopericardium (1 2, 8, 9 14)

Nearly all deaths in connection with c.c. have occurred in poor lives," mostly children suffering from severe, congenital heart disease. That fatal haemopericardium may complicate right c.c. even in a normal heart appears from the following case report.



Fig. 3. The heart partly opened with the catheter replaced in the perforation in the anterior wall of the right ventricle.

Case report

April 1962, 59-year-old former fisherman as admitted to medical department for chest diseases with diagnosis of *asthma*, but with special view to evaluation for disablement compensation.

The patient had suffered from periodic, progress. *bronchitis* since 1944, but had never had *real asthmatic attacks. Repeated radiog*raphy of the chest and nasal sinuses, bronchography and bronchoscopy ECG examinations of sputum and blood, estimation of electrolytes, etc. had been uncontributory. Intracutaneous tests for allergy (1958) indicated sensitivity to house dust, animal hairs, feathers and threshing dust. In 1961 the maximum breathing capacity was 45 l (60 l after propynalin) vital capacity 3 l, and residual olume 38 of the total capacity

Previous treatment had included ephedrine, pracol, phenergan, propynalin, durabolin, various antibiotics and cortisone during recent months ledercort 2 mg × 2 daily

During the last weeks coughing, expectoration and some dyspnoea had incapacitated the patient. He had therefore given up his work at sea and was now employed in nautical shop

On admission the general condition was good, but there was slight asthmatic dyspnoea and diminished breathing sounds. Physical examination was otherwise uncontributory chest radiography and ECG were found to be normal. Hb was 97 differential count was normal with 1 % eosinophils. The sputum contained Candida albicans, nonhaemolytic streptococci, Gram-negative cocci and Pfeiffer bacilli, sensitive to the common antibiotics. Arterial oxygen saturation was 93 %. Ledercort, ephedrine, aecophylline, phenergan and chloromycetin spray were given, and the condition rapidly improved.

On May 1 *right cardiac catheterization* was performed to record the pressure in the pulmonary artery. A teflon catheter was easily passed from the left cubital vein into the right ventricle under fluoroscopic control. According to the case record, the tip of the catheter was then gently advanced into the (supposed) pulmonary artery without undue resistance or untoward reactions. A radiograph was taken (fig. 1) the pressure recorded (fig. 2) and the catheter slowly withdrawn. When the tip was in the superior vena cava, the patient suddenly became ill and vomited. The catheter was completely withdrawn (30 min. after the introduction) and the patient returned to bed. Soon afterwards he complained of pain in the chest, he was sweating, felt cold, and the pulse was weak. Cedilanid, 2 ml intravenously and nicethamide, 2 ml subcutaneously were given twice, and oxygen was administered. Death ensued 2 hours after the onset of symptoms.

As the death might be attributed to the diagnostic procedure performed, the case was reported to the police. A complete medicolegal autopsy was ordered and performed by the author

Autopsy. The body was that of a strongly built, normally nourished man, 177 cm, 80 kg.



Fig. 1 Roentgenogram displaying the catheter partly in the pericardial sac.

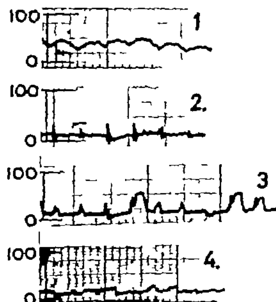


Fig 2 Pressure curves recorded at four positions during withdrawal of the catheter from a suspected position in the right pulmonary artery to the right atrium. Recording no. 4 may be from the right atrium, but recordings no 1—3 are without doubt from the pericardial sac, nos. 2 and 3 showing irregular pressure oscillations at a low mean pressure level.

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rather rigid. In spite of the recorded information that no unusual resistance was felt during the catheterization, it is evident that too much pressure had been exerted. It must also be stressed that continuous electrocardiographic monitoring was not used during the investigation.

At autopsy the right ventricle was slightly dilated with a fairly thin wall, but general experience, including subsequent observations by the author shows that a thickness of 2 mm of the right ventricular wall is not at all rare. Hypothetically it was suggested that the preceding treatment with cortisone might have weakened the myofibrils. Histological examination, however revealed no visible evidence of degeneration the myocardium was quite normal.

Nearly all previously reported fatalities during cardiac catheterization have occurred in severely ill patients, in whom the indication for catheterization was usually a vital need of a detailed anatomical diagnosis prior to possible surgical intervention. In some reported fatal cases it was difficult to decide whether the catheterization or the basic disease caused the death. Cases in which sudden death occurred shortly *before* a planned catheterization prove how easily death may falsely be ascribed to a diagnostic or therapeutic procedure. In cases with cardiac tamponade due to perforation, however it is obvious that the catheterization is the cause of death

In the reported case the patient was not severely ill Although suffering from chronic bronchitis, he was able to manage his new job, and the disease itself hardly indicated the performance of a cardiac catheterization. This was only carried out to obtain a more detailed functional evaluation of his condition than otherwise possible for insurance purposes.

The Danish Medicolegal Council, represented by two cardiologists, a radiologist and a forensic pathologist, considered that the catheterization had been properly indicated, classified the fatal perforation as accidental and criticized only the observation and treatment of the shock. This involved that no legal negligence was exerted, and no charge was brought against the examiner

In the opinion of the author cardiac catheterization — even right heart catheterization — should be restricted to cases with an obvious and severe clinical indication, and should be carried out only in departments equipped for the detection and treatment of possible complications.

Summary

Some hazards involved in heart catheterization, in particular the risk of cardiac perforation with haemopericardium, are reviewed. Most previously reported fatalities occurred in extremely bad lives

A detailed report is given of the case of a 59-year-old man with chronic asthma bronchitis, but without heart disease, in whom perforation of the right ventricle with fatal tamponade occurred during catheterization. The death was ruled as accidental and no charge of negligence was brought against the examiner

In the author's opinion even right heart catheterization should be restricted to special departments equipped and prepared to treat possible complications.

Acknowledgement

My thanks are due to H. Goetzsche, M. D., Cardiological Laboratory Århus Municipal Hospital, who kindly looked over the manuscript from a cardiological point of view

External examination was uncontributory apart from injection marks in the left cubital fossa. The internal examination included an initial control for air embolism. Instead of this, a tremendous *haemopericardium* was revealed, with 500 ml of partially coagulated blood causing tamponade of the heart. The blood originated from a *perforation in the anterior wall of the right ventricle* 2 cm above the inferior and 1.5 cm to the left of the right margin of the heart (fig 3) which was of normal size, 12 × 12 cm, 360 g Both atria and the left ventricle were normal, the right ventricle appeared slightly dilated, but not hypertrophic, measuring 2–4 mm in thickness. No signs of disease was seen adjacent to the perforation or elsewhere in the myocardium.

The lungs displayed a moderate degree of emphysema. The tracheobronchial tree contained tough mucous, several bronchi were slightly dilated and the mucosa was red. Each of the adrenals (27 g together) contained a yellow 1 0–1.5 cm cortical node. There was hardly any atheromatosis.

Histological examination revealed normal structure of the myocardium moderate pulmonary emphysema, chronic bronchitis and simple cortical adrenal adenomata. The remaining organs revealed normal structure.

Legal considerations

The cause of death was stated to be cardiac tamponade by haemopericardium, due to right ventricular perforation during cardiac catheterization (N 997) The mode of death was ruled as *accidental*, complicating a non therapeutic medical procedure (E. 946)

The prosecution submitted the case to the Medicolegal Council asking whether mistakes, faults or negligence had been committed by the examiner The council replied No criticism could rightly be directed against the indication for the examination and the introduction of the catheter has apparently been performed in the usual way During the passage of the catheter through the right ventricle, the wall was perforated and further advance of the catheter occurred in the pericardial sac. The perforation must be considered extremely rare, but accidental.

On the radiograph (fig 1) the perforation is not unquestionably visible, although the course of the catheter gives rise to a strong suspicion of perforation. The state of shock which occurred after the removal of the catheter is not an unequivocal sign of perforation of the heart'

The council concluded that the examiner had not been negligent through failure to diagnose the perforation, whereas the observation and treatment of shock following the examination had been insufficient.

The National Health Service and the prosecution adopted the opinion of the Medicolegal Council and no charge was brought against the examiner

Discussion

It is noteworthy that in both previously reported cases and in the present case the perforation of the right ventricle occurred without any immediate symptoms or signs. No unusual resistance was felt during the alleged gentle passage of the catheter and no reaction was observed in the patient who was fully conscious. The recording of the pressure in the supposed pulmonary artery was completed (cf. fig 2) and only after the autopsy was it realized that the measurements had actually been performed with the catheter in the pericardial sac. Neither did the fluoroscopic examination immediately disclose the fatal error although reappraisal of the radiograph (fig 1) after autopsy clearly disclosed that the catheter had surpassed the limits of the heart The symptoms started when withdrawal of the catheter left the perforation open to produce a haemopericardium. Unfortunately this serious complication was not diagnosed in time, and no relevant therapeutic measures were attempted It should be added that there is no surgical department in the hospital concerned

The cause of the perforation is not quite clear The catheter was, however a new Cournand type teflon catheter (USCI type T 50 8F) which is known to be

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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A Comparative Study of the Diuretics Chlorthalidonum and Cyclopenthiazidum

By

E. J. Dorhout Mees and G. G. Geyskes

The physician who wishes to prescribe a diuretic today finds himself confronted by a wide range of agents from which to take his choice. Although parenteral mercury compounds, if judiciously applied, are still regarded as the most effective (8) the preference in more protracted ambulatory treatment is for an oral compound.

Since the introduction of chlorothiazide numerous agents with a similar action have been marketed. Of course each compound is described as having more advantages than the others. Obviously however such differences as exist are primarily based on difference in the quantity of the substance required to ensure a certain effect (i. e. natriuresis). The differences in activity are believed to be based primarily on the difference in fat solubility and related differences in absorption and distribution in the organism (12). It is probable that the maximum effect to be obtained by increasing the dosage is not widely different for the various agents. In more protracted therapy moreover the question whether a drug has few side-effects is more important than the question whether maximum dosages perhaps produce a slightly more distinct effect.

The fact that diuretic medication yields such favourable results, particularly in the treatment of moderate hypertension, warrants the expectation that many patients will receive treatment with these agents for considerable periods of time.

It seemed of interest, therefore, to establish whether agents with a comparable natriuretic effect might differ in side-effects, in view of which a given drug would be preferable.

To establish this we made comparative studies of chlorthalidone (Hygroton® Geigy) and cyclopenthiazide (Navidrex® Ciba) which, on the basis of available data, could be expected not to be inferior and perhaps even to be slightly superior to some of the older agents (1, 6).

Apart from side-effects in the sense of allergic reactions (which are seldom) there are a number of effects inherent to the action of the agent on the renal tubules, viz

Submitted for publication November 18, 1963.

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Table II. Comparison of effects on K⁺ and Na⁺ excretion of comparable dosages of the two drugs

Basal Na excretion (mEq day)	Substance	Drug 1 (mg)	Drug 2 (mg)		n_2	u	P
>10	K	Hyg 50	Na 0.5	5	4	8	0.73
<10	K	Na 0.5	Hyg 50	3	11	8	0.23
	K	Na 0.5	Hyg 50	7	16	51	0.77
>10	K	Hyg 200	Nav 2.25	3	5	6	0.79
<10	K	Nav 2.25	Hyg 200	11	7	35	0.79
	K	Nav 2.25	Hyg 200	16	10	77	0.90
>10	Na	Hyg 50	Nav 0.5	3	4	9	0.90
<10	Na	Nav 0.50	Hyg 50	5	11	15	0.88
	Na	Nav 0.5	Hyg 50	7	16	33	0.87
>10	Na	Hyg 200	Na 2.25	3	5	5	0.57
<10	Na	Hyg 200	N 2.25	7	11	26	0.29
	Na	Hyg 200	Nav 2.25	10	16	58	0.26

ing the same patient with both agents while on both occasions his condition is completely identical. Apart from this, our purpose was to find an agent enabling us to keep hypertensive patients constantly at a mild degree of salt depletion.

Material and methods

The test subjects were hypertensive patients and obese patients, none of whom showed any sign of cardiac decompensation or renal insufficiency. During the test, all subjects received the standard low-salt diet of our hospital, which contains some 30 mEq Na per day, the composition of which was kept as constant as possible. Both agents were tested at "high" as well as at "low" dosage after establishing, from the literature and preliminary experiments, that the dosages chosen had approximately the same natriuretic effect: the high dosage closely approached the maximum effect, whereas the "low" dosage, although unmistakably active, remained considerably below this effect. One of the two agents, moreover, was also tested at medium dosage (Navidrex®). Every test period was preceded by 3 control days, the test periods covering 4 days each. Navidrex was given during 4 days at a dosage of 2 × 0.25 mg daily ("low") or 3 × 0.75 mg daily ("high") and 2 × 0.5 mg daily ("medium"). Because of its long-term action, Hygroton® was given only during the first 2 days of the test period, at a dosage of 2 × × 25 mg daily ("low") or 2 × 100 mg daily ("high"). Attempts were made to ensure that the same patient was submitted to as many tests as possible, the control days of subsequent test period beginning immediately upon completion of test period. The two agents were used alternately and successive patients were alternately started on the one drug or the other.

A total of 63 test periods were completed in 22 patients. A number of periods had to be *eliminated* because of *unexpected* departure, and errors on the part of the patient, the nurses or the laboratory, the schema designed was consequently not always maintained. Four patients were submitted to only one test period, 2 other patients completed 10 and 11 test periods, respectively. The remaining 16 patients were each tested 3 times.

Table 1 Comparison of the effect on K^+ and Na^+ excretion of different dosages of each drug

Basal-Na excretion (mEq/day)	Substance	Drug 1 (mg)	Drug 2 (mg)	n_1	n_2	n	P
>10	K	Nav 0.5	Nav 1	4	2	6	0.27
<10	K	Nav 0.5	Nav 1	3	8	1	0.994
	K	Nav 0.5	Nav 1	7	10	23	0.39
>10	K	Nav 1	Nav 2.25	2	5	6	0.43
<10	K	Nav 1	Nav 2.25	8	11	72	0.01
	K	Nav 1	Na 2.25	10	16	170	0.02
>10	K	Hyg 50	Hyg 200	5	3	10	0.29
<10	K	Hyg 50	Hyg 200	11	7	72	0.001
	K	Hyg. 50	Hyg. 200	16	10	130	0.004
>10	Na	Nav 0.5	Nav 1	4	2	3	0.73
<10	Na	Nav 0.5	Na 1	3	8	6	0.90
	Na	Nav 0.5	Nav 1	7	10	18	0.96
>10	Na	Nav 1	Nav 2.25	2	5	8	0.19
<10	Na	Nav 1	Nav 25	8	11	70	0.02
	Na	Nav 1	Nav 2.25	10	16	124	0.01
>10	Na	Hyg. 50	Hyg. 200	5	3	15	0.02
<10	Na	Hyg 50	Hyg 200	11	7	63	0.01
	Na	Hyg 50	Hyg 200	16	10	134	0.002

a. Increased potassium excretion with a decrease in blood potassium concentration and potassium depletion in the cells.

b Increased chloride excretion relative to sodium excretion, giving rise to hypochloraemic alkalosis in the blood.

c. Influences on the uric acid metabolism resulting in an increased blood concentration

d. An influence on the carbohydrate metabolism has also been described this can cause exacerbation of an existing diabetes, and possibly cause manifest diabetes in some cases where none existed thus action however is infrequent (10 13)

Effects a) and b) are capable of mutual synergism. Whether the anomaly mentioned under b) is harmful, has so far remained obscure.

We confined our study to determination of the urinary excretion of Na^+ K Cl NH and uric acid of the blood concentration of Na^+ K Cl HCO and uric acid, and of the concentrations of creatinine and urea in urine and blood.

Since both the Na and the K expelling action is largely dependent on the patient's condition it was considered less suitable to study the action in oedematous patients. For in that case there would be virtually no possibility of treat

Table III. Effects of the drugs on electrolyte excretion, calculated in different ways (see discussion in text)

Mean Na excretion/24 hrs on control days	No. of observations	Dose (mg)	Mean excretion in mEq/24 hrs								Absolute increase during test days as against control days in mEq/24 hrs			Increase[1]	
			Two control days				Four test days								
			Na	K	K/Na	Cl	Na	K	K/Na	Cl	Na	K	Cl	K	Cl
<10	8	Nav 1	2.5	28.4	11.4	11.3	11.4	39.5	3.5	50.2	9.1	11.1	18.9	1.2	2.0
	10	Hyg 50	2.7	35.5	13.1	18.9	16.1	55.2	3.4	36.1	13.4	19.7	17.2	1.5	1.3
	11	Nav 2.25	3.9	33.2	8.5	14.9	38.4	71.5	2.0	72.9	32.5	38.3	58.0	1.2	1.8
	8	Hyg 200	4.5	41.1	9.6	13.3	39.4	82.2	2.1	64.3	35.1	41.1	51.0	1.2	1.4
>10	2	Nav 1	19.5	45.1	2.3	18.1	38.7	73.8	1.9	79.7	19.2	28.7	61.6	1.5	3.3
	5	Hyg 50	19.2	43.8	2.3	32.1	43.5	67.6	1.6	90.3	24.5	23.8	58.2	1.0	2.4
	3	Na 2.25	22.3	47.3	2.1	13.8	81.9	94.3	1.2	117.2	58.7	47.0	103.6	0.8	1.7
	3	Hyg 200	49.5	40.7	0.8	60.9	123.2	80.0	0.7	135.8	73.7	39.3	74.9	0.5	1.0

[1] Increase (in K and Cl^- respectively) in mEq/mEq increase in Na

The ratio K/Na excretion before and during administration is analysed in table III. This table does not include the data on all subjects, but only on those whose basic Na and K excretions did not deviate by more than 30 % from the means given in the table. For the range of deviation of these basic values was considerable, particularly in the group with Na excretion exceeding 10 mEq/day; this would have further impeded evaluation of the significance of the changes.

In addition to the quotient excreted K/excreted Na, the absolute value and the increase in excretion of each of the ions is also indicated. We also calculated how many mEq of K and Cl above the control value were excreted under the influence of the agent, as against every mEq Na expelled extra. The last mentioned finding is the crucial datum in practice because it indicates the true ratio between desired and undesirable effect.

We see that, at the "low" dosage of each drug, the K/Na quotient on the control days at low Na excretion is very high, while it shows a marked decrease after medication. At a higher Na excretion on the control days, the K/Na quotient is of course much lower and changes only slightly after medication. In the latter case the drugs none the less increase the K excretion even more, although in both cases the quantity of K expelled per mEq Na is about the same.

There is an unmistakable difference between the "low" and the "high" dosage in terms of K and Cl excretion per quantity of Na expelled, but only in those cases which had shown a relatively high control Na excretion (not when this had been very low). We intend to return to this in the discussion.

Even without statistical elaboration it was seen that no prominent differences between the two agents existed. After Na idrex, it is true, there was a relatively

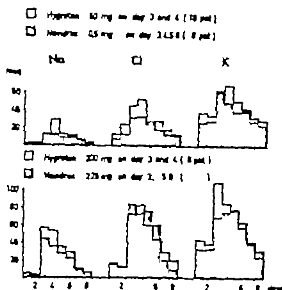


Fig. 1 Mean electrolyt excretion before, during and after drug administration. The results of both drugs, though obtained at different times, are plotted simultaneously at the comparable days.

Results

Because the Na^+ excretion during the control period gives an indication of a patient s salt depletion at that time, the results in terms of electrolyte excretion for each dosage and each agent were divided into a group of data concerning a "basic excretion of Na less than 10 mEq/24 hours, and a group in which it exceeded 10 mEq/24 hours.

Table I presents the results of a statistical study into the significance of differences in effect upon the Na and the K^+ excretion, respectively caused by different dosages of the same agent. As a criterion of action we accepted the difference in mean Na and K^+ excretion, respectively between the test period and the preceding control period When comparing two different dosages the Wilcoxon test was applied (as in table II) to the difference in Na^+ and K excretion respectively between the test period and the control period

The statistical department of the central organization T N O which carried out the statistical elaboration, in this respect remarks that the range of deviation of the various control periods and test periods of one agent was considerable, and that the reliability of results would be enhanced by increasing the number of test subjects.

It was found that no significant difference exists between the influence on Na^+ and K^+ excretion of 0.5 mg and that of 1 mg Navidrex per day but there is a difference between 1 and 2 25 mg Navidrex (but for the Na excretion this was only seen in patients who had a low basal Na excretion) and also between 50 and 200 mg Hygroton.

In the same way as table I, table II presents a comparison of the effects of the two agents tested It shows that there is no significant difference between the low dosages of the two agents, nor between the high dosages of the two agents.

To give an impression of the course of the Na^+ K^+ and Cl excretion before, during and after administration of the diuretic we plotted the graph presented in fig 1 The observations during administration of a comparable dose of each of the agents tested were always plotted in the same figure, and this was done separately for the low and for the high dosage of each of the agents.

As could be expected the effect of the longer-acting (and therefore accumulating) Hygroton though most marked on the two days of administration was still unmistakable during the two days without Hygroton administration

On the first control day after completion of the test, both agents still exerted a slight influence on the electrolyte excretion

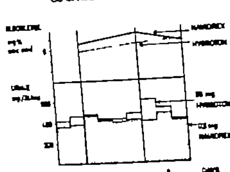


Fig. 2. Mean uric acid blood level and excretion during "low" drug dosage.



Fig. 3. Mean uric acid blood level and excretion during "high" drug dosage.

Table V. Mean plasma concentration of creatine and uric acid with and without diuretic therapy

Diagnosis	Men			Women		
	No.	Creat. (mg%)	Uric acid (mg%)	No.	Creat. (mg%)	Uric acid (mg%)
No hypertension	12	0.9	4.8	10	0.7	4.5
Hypertension without diuretics	6	1.1	6.5	13	1.0	5.9
Hypertension and						
Chlorothiazide	7	1.5	7.7	10	1.0	6.7
Hydrochlorothiazide	5	1.2	6.8	18	0.9	6.9
Chlorothalidon	6	1.3	7.8	12	0.9	6.5
Cyclopenthiazide	4	1.1	7.1	7	1.1	6.9

In the course of follow-ups on hypertensive patients at the out patient clinic, we also made a number of uric acid determinations in patients using various diuretics (table V).

Because of the wide range of standard deviations, no statistical comparison was carried out. The impression gained from these findings, however, confirms the conclusion formed by others (4) that there is no difference in influence on the uric acid concentration. We found, however, that the uric acid concentration is as rule somewhat higher in hypertensive patients, even when no diuretics are given. It has not been established whether this correlates with a slight disturbance in renal function (in view of the high creatinine values) or with Na uptake (4) or with yet another cause.

Discussion

General considerations

In evaluating the influence of diuretics on the excretion of various electrolytes, we encounter a number of typical difficulties.

In addition to problems of individual variation and imponderable influences involved in any investigation of drugs, which can be overcome by correct statistical elaboration of sufficiently exhaustive data, there is the difficulty that

Table IV Mean plasma concentration before and after the four test days in mEq/l

Basic Na exc.	Agent	Sodium				Potassium				Chloride				Bicarbonate			
		No. of periods	Before	After	Difference	No of periods	Before	After	Difference	No. of periods	Before	After	Difference	No. of periods	Before	After	Difference
>10	Nav 0.5	4	147	143	−4	4	5.0	4.1	−0.9	4	101	94	− 7	4	21 7	23.9	+2.2
	Hyg. 50	3	144	139	−5	3	4.3	3.7	−0.6	3	102	93	− 9	3	19.9	6.3	+6.4
	Nav 2.25	5	141	141	0	5	4.2	3.6	−0.6	4	92	90	− 2	3	30.6	31 7	+1.1
	Hyg. 200	2	139	139	0	2	4.6	3.2	−1 4	3	107	94	−13	3	22.3	26.8	+4.5
<10	Nav 0.5	1	140	143	+3	1	3.2	3.3	+0 1	1	90	88	− 2	5	23.3	26.0	+2.7
	Hyg 50	5	143	140	−3	5	4.3	3.7	−0.6	5	97	94	− 3	5	27 1	28.7	+1.6
	Nav 2.25	10	140	136	−4	9	4.2	3.6	−0.6	9	98	94	− 4	8	25.8	28.9	+3.1
	Hyg. 200	5	137	139	+2	5	4.3	3.6	−0 7	5	96	94	− 2	5	7.3	7 6	+0.3

more intensive Cl excretion particularly in the group with a high control Na^+ excretion But we do not wish to attach too much significance to this, particularly because we saw no marked differences in serum concentrations (table IV) following administration of each of the drugs.

The NH_4^+ creatinine and urea excretions were also determined in all patients. The data are not presented because they revealed no distinct differences during administration of the test substances.

Table IV presents the concentrations of Na^+ K^+ Cl and HCO_3 before and at the end of the "period of administration We find no unmistakable changes in Na^+ concentration but there is a decrease in K^+ and Cl concentrations and an increase in the HCO concentration

There is no striking difference between the effects of the two drugs on these results the dosage, too seemed to make relatively little difference. In this respect it must be pointed out, however that the "control values as a rule already deviate from the normal (and to varying degrees) as a result of medication during preceding periods.

Because the changes in the serum electrolyte concentrations are known not to be progressive during more prolonged administration, it is obvious that little or no influence can be expected from medication at an already deviating initial value.

Uric acid metabolism

The urinary excretion of uric acid was subject to considerable variation from day to day even during the control period After administration of each of the drugs the blood concentrations always increase. Fig 2 indicates both the course of the 24-hour excretion and the blood concentration before, during and after administration of each drug

A striking feature is that there is no distinct change in excretion during administration of each drug despite an increase in blood concentration For this reason we refrained from statistical elaboration of these data.

last column) at a higher basic Na^+ excretion. This possibly affords an explanation of the above mentioned discrepancies in the literature, because most of these tests were carried out at even higher basic excretions. These publications, moreover always concerned a single dose, while we used fractional doses distributed over the day for the long acting Hygroton this does not make such of a difference, but for Navidrex it does.

The last column of table III shows that, at high Na excretion during the control period, the Cl excretion exceeds that of Na at low dosage much more than at a high dosage. This must probably be ascribed to the carboxylase-inhibiting properties of these agents, which assume a more important role at a high dosage. We have pointed out that the two drugs probably differ in this respect also but the much more pronounced influence of the basic Na excretion illustrates again how many precautions must be taken before attributing a given effect to a given drug These differences are of minor clinical importance.

The K excretion

The K excretion caused by a diuretic is to at least the same degree dependent on the condition of the organism as is the Na excretion. The degree of Na depletion determines, via the aldosterone production, the tendency towards K loss under the influence of any stimulus promoting Na excretion, so that some even regard this as the sole cause of K loss (5) It is obvious that the K concentration of the organism and the acid-base balance (15) are also factors determining the degree of K loss.

In comparing the effects on K excretion of tw drugs, therefore, even greater prudence should be observed than in regard to NaCl excretion. It is true that some investigators (11) saw less K^+ excretion after hydrochlorothiazide than after chlorothiazide, while others saw less K excretion after Navidrex than after chlorothiazide (1) clinically however the difference is seldom appreciable (8)

Reporting percentage increases without presenting absolute figures (1) greatly impedes a critical evaluation of papers. Ford's report (7a) stating that, after 6 months of continuous therapy the K / Na ratio in the urine following Hygroton was nearly one-third of that following Chlotride, cannot be interpreted either without more detailed data. Our study yielded no statistically significant differences. In table III the lack of reliability of the K /Na excretion factor is once again demonstrated.

At a low basic Na excretion, the quotient following a low dose of each of the drugs was 1 1/2 times as high as that following a high dose. Yet the quantity of K expelled per mEq Na was virtually the same (last column but one) At a higher basic Na excretion an even lower quotient was found, again with equivalent K expelled after a low dose. After a high dose, however an unmistakable predominance of the natriuretic over the kaliuretic effect seems to occur

The question whether more ample salt uptake reduced the K loss after diuretics, cannot be answered with certainty on the basis of our findings. We observed that, at least t a high dosage, less K is lost per mEq Na expelled (table III comparison between high and low basic Na excretion) It is very well possible, however that t a higher salt uptake, despite greater natriuresis, the same degree of salt depletion is nevertheless not reached,

the effect of a drug administered is largely determined by the "condition" of the organism. Quite apart from the influence of a change in the disease per se on this "condition" the drug administered provokes (in normal subjects also) reactions of the normal homeostatic mechanisms governing the electrolyte balance.

These counterreactions are both humoral and haemodynamic in character. Thus NaCl depletion — even when caused by preceding administration of a diuretic — provides a stimulus to Na^+ retention which partly involves the hormone aldosterone. An increased production of this hormone in certain conditions stimulates K^+ excretion. But this effect is not manifested when the urinary Na^+ excretion is low; it is, however, when this is high in pathological conditions. This, therefore, occurs, not in secondary but in primary aldosteronism, probably also in salt losing conditions and when Na^+ excretion is forced by a diuretic. Thus a mercury diuretic, while inhibiting the K^+ excretion in some circumstances (2) may at other times cause an increase in K^+ excretion (14).

The NaCl excretion

Without going into detail about the exact mechanism of renal electrolyte excretion, it can be stated on the basis of general homeostatic considerations that the organism is more readily forced to excrete a given electrolyte to the extent to which it is already excreting large quantities of this electrolyte (in response to increased supply). It is therefore of only limited importance to study the action of a diuretic (as is usually done in animal experiments) during salt infusion; presentation of an Na^+/K^+ quotient obtained under these conditions in the urine imparts even less information. Thus it may occur that a certain drug is very efficient to increase Na^+ excretion in less severe patients, while another drug is more apt to enforce in Na^+ diuresis in case of very strong Na^+ retention.

As an example, we mention an observation by Stewart et al. (14). These investigators found that, in some patients, Hygroton caused a higher Na^+ excretion than Mersalyl whereas in "resistant" patients the latter caused more Na^+ excretion than the former. The same applies to a comparison of various dosages of the same agent. Thus Ford found that a dose of 200 mg Hygroton produced a hardly more marked natriuretic effect than 50 mg (7), which seems completely contradictory to our observations. Truniger reported a virtually maximal action of Navidrex at a dosage of 1 mg (15).

With hydrochlorothiazide, too, other authors saw virtually no difference in natriuretic effect between 25 and 100 mg when studying the substance during a period of high salt uptake.

Ford found that 25 mg hydrochlorothiazide in a certain test arrangement was more effective than parenteral mercury compounds (6), from which he concluded that this dose was as effective as 2,000 mg chlorothiazide. All these observations are in no way compatible with the experience of many clinicians.

For this reason we have imitated Veyrat (17) in that we divided our results into two groups, i. e. a group with very low Na excretion (< 10 mEq/day) during the control period, and a group with over 10 mEq/day excretion.

Both table I and table III indicate an unmistakable difference in natriuretic and chloruretic effects between the high and the low dosage of each of these agents. We also see that this difference is less significant, e. g. not significant (table I

last column) at a higher basic Na ex cretion. This possibly affords an explanation of the above mentioned discrepancies in the literature, because most of these tests were carried out at even higher basic excretions. These publications, more over always concerned a single dose, while we used fractional doses distributed over the day for the long-acting Hygroton this does not make such of a difference, but for Navidrex it does.

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so that the more favourable K^+/Na^+ quotient is also associated with a less favourable therapeutic effect.

The uric acid metabolism

In view of the exhaustive study published meanwhile by Bryant et al. (4) which entirely in accordance with our experience, revealed no significant difference between chlorothiazide, hydrochlorothiazide and Hygroton it seemed unlikely that Navidrex would show a different behaviour in this respect.

The influence of the abovementioned agents on urate excretion is a complex one at a high intravenous dosage they sometimes have a uricosuric action (4) Most authors mention a reduced clearance as a cause of increased blood concentrations. The exceedingly marked variation of the 24-hour secretion discouraged us from calculating a uric acid clearance. The temporary diminution of excretion to be expected on the first day in our set up was often absent we even raised the question whether increased uric acid production in some patients might be a partial cause of the increased concentration.

Duration of action of test substances

Although no significant differences between the two test substances were found either in influence on Na K+ and uric acid excretion or in any of the other magnitudes measured, there was a remarkable difference in duration of action and in the activity of the marketed tablets.

We compared the action of 1/2 tablet Hygroton (100 mg per tablet) daily for 2 days with that of 1 and 2 tablets Navidrex (0.5 mg per tablet) respectively for 4 days ("low dosage) and 2 days of 2 tablets Hygroton with 4 days of 4 1/2 tablets Navidrex. It is not surprising therefore, that Bryant et al. (3) at a daily dosage of 200 mg Hygroton found a more pronounced antihypertensive effect than at 750 mg chlorothiazide, which latter dose has the same effect, according to Ford (7) as at best 25 mg Hygroton.

We did not consider the action on blood pressure (which asserts itself only after more prolonged medication) it is probably in some way linked to the natriuretic action

We found no fundamental advantages of one of the test substances over the other It did become clear however that the commercial tablets of chlorthalidone contain relatively much greater quantities of active principle than any other diuretic. In the case of long term maintenance therapy moreover the long duration of action (3 days) must be taken into account whenever an intermittent action is desired (advocated by many to avoid K^+ depletion) at least 3—4 consecutive days without medication must be planned.

The choice of drug will be determined largely by the question as to whether a protracted action is desired.

Summary

The Na^+ K^+ and Cl expelling action and the influence on uric acid metabolism of two oral diuretics, cyclopenthiazide (Navidrex®) and chlorthalidone (Hygroton®) were compared by alternate administration in non-oedematous patients during strict salt restriction.

When used in comparable dosages, the two substances showed no significant differences. Mention is made of the importance of the degree of salt depletion of the organism at the time of administration in view of an evaluation of both the natriuretic and the kaliuretic effect.

A number of seemingly contradictory reports in the literature are discussed.

Although a carefully designed schema of dosage revealed no different influences on the electrolyte values in urine and blood, there was a distinct difference in duration of action and activity per tablet between the two substances. Hygroton has a much more protracted action, and the effect of one 100-mg tablet is much more intensive than that of one 0.5-mg tablet of Navidrex.

It is these characteristics which partly determine the choice of substance for a given patient.

Acknowledgement

This investigation was supported by a grant from the Clinical Drug Research Organization T.N.O. and the statistical elaboration was carried out by the Data Processing Department of this organization.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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The Value of Lung Physiotherapy in the Treatment of Acute Exacerbations in Chronic Bronchitis

By

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The course of chronic bronchitis is characterised by acute — often seasonal — febrile exacerbations. These attacks show two clinical aspects: firstly they appear as acute febrile episodes with the characteristic general symptoms of infectious illness, and secondly they affect to a greater or lesser extent the function of the lungs, in a similar manner to that in which other exudative or space occupying lesions in the thorax can compromise the respiratory function.

Today there are a number of possible methods of treating both the infectious process itself and also the change in respiratory function, if this occurs. With regard to the former, penicillin, the tetracyclines, and sulphonamides have all been shown to be useful (1, 3, 4, 5, 6, 7).

Where the acute respiratory insufficiency occurring in the course of chronic bronchitis is concerned, in the most severe cases of reduced oxygen saturation and hypercapnia with respiratory acidosis, tracheotomy and oxygen therapy with artificial ventilation have been used (2).

In the *mild* cases of exacerbation of bronchitis it is generally sufficient to treat the infection. In the *severe* cases there are vital indications for a combination of antibiotic therapy and tracheotomy with artificial ventilation. Between these extremes there is a residuum of cases in which the respiratory function is affected, but not to such an extent that there is indication for tracheotomy etc. In these cases the "mechanical" element of the illness is often an indication for the use of oxygen in small doses correlated with the arterial blood chemistry. Occasionally this form of treatment is, however, found to be insufficient, particularly when part of the airway is blocked by excessive secretions in the bronchi.

The so-called lung physiotherapy, which includes advice on the full utilization of the available respiratory potential, assisted coughing, positional drainage, etc., is widely used, not least in surgery of the thorax, as prophylaxis against or treatment of "peripheral" and more "central" obstructions of the respiratory tract.

Because of the similarities in respiratory problems between on the one hand this type of newly operated patients with

Submitted for publication November 20, 1963.

Table I Data of age and sex distribution together with the occurrence of complicating disease in the material comprising 68 acute episodes in 63 patients with chronic bronchitis

	Treated group		Control group	
Sex	♀	♂	♀	♂
No. episodes	12	23	12	21
Average age (yrs)	56	63	54	64
Occurrence of complications				
Chr cor pulmonale	7		9	
Dependent oedema	4		6	
Adiposity	1		2	
Thorax deformity	3		2	
? Boeck's sarcoid	2		1	

Table II Change in arterial blood chemistry in the course of the illness in the patients with obvious respiratory insufficiency (oxygen sat ≤ 80 % and/or pCO_2 ≥ 45 mm on first estimation) Number of observations in brackets

	Treated group	Control group
No. patients	16	14
O saturation (%)		
1st estimation	72 (15)	74 (12)
2nd estimation	77 (12)	80 (13)
pCO (mm Hg)		
1st estimation	53 (16)	52 (13)
2nd estimation	44 (15)	48 (13)

a tendency to accumulate secretions in the respiratory tract and on the other bronchitics suffering from an acute attack superimposed on their chronic disease lung physiotherapy has been used in the treatment of the acute, febrile exacerbations of chronic bronchitis. As no evidence in support of the effect of such physiotherapy appears to have been published the investigation described below was planned

Material and methods

A total of 69 patients are included in the investigation, being those admitted to the medical departments B and F in the period 1 I 1961—1 IV 1963 in whom the diagnosis of an acute flare-up in chronic bronchitis was reached immediately following admission.

This patient material does not represent all the patients who were discharged with this diagnosis during the same period, as in some cases the diagnosis was reached too late for the patient to be included in the study

The diagnosis of acute exacerbation in chronic bronchitis was made in patients who had had a cough for a longer period of time (at least 6 months) and in whom there was acute deterioration with raised temperature and muco-purulent expectorate.

All patients in whom the diagnosis was reached sufficiently early and whose illness corresponded to the above definition, have been included regardless of the presence of complications such as chronic cor pulmonale, other forms of cardiac insufficiency deformities of the thoracic cage etc. It was, however necessary to reject 6 of 69 patients in 3 the investigations were incomplete, 1 died on the third day after admission, and in 2 cases malignant disease was demonstrated during admission. Of these 6 patients 3 were from the control group.

The treatment of the patients followed the conventional lines antibiotics (as a rule sodium benzyl-penicillin (penicillin-G) 1 mill. units twice daily) bed-rest, and if necessary digitalis, theophylline, diuretics, expectorants, and oxygen. The only difference in the treatment of the patients in the two groups was that those patients admitted on even dates received daily lung physiotherapy whilst those admitted on odd dates did not receive this additional treatment. Very occasionally it was found advisable for psychological reasons to give lung physiotherapy to patients who according to the above rule should not have received it these patients have been included in the treatment group These patients were those who had received lung physiotherapy during a previous admission. By lung physiotherapy we mean expansion exercises "tapotement" or "vibrations" and postural drainage, administered daily for 10 days in the ward by a specially trained physiotherapist. Later in the

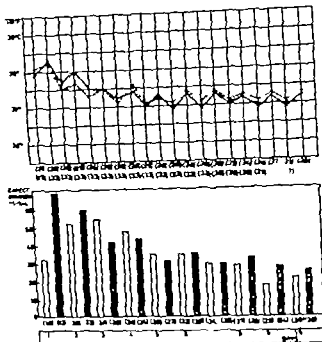


Fig. 1 The average course of temperature and average daily amount of expectorate in the two groups. Treated group solid line in temp. graph, white columns; control group: broken line in temp. graph, dark columns. Figures in brackets indicate No. of observations; in the temp. graph those for treated group are given above those for control group.

course of the illness, in general after the investigation period, some patients were transferred to breathing exercises in groups.

In the 63 remaining patients there were total of 68 acute episodes (admissions) divided into *treated* group (+ lung physiotherapy) and control group (— lung physiotherapy). The distribution according to sex, average age, and occurrence of complicating disease can be seen from table I. *Two patients are included in* both groups, they are represented by at least one episode in both treatment and control group.

As basis for assessment of the effect of therapy the daily temperature and amount of expectorate (the measurement of expectorate being carried out by the same person) has been used; in addition analyses of the arterial blood at the beginning and end of the period of treatment, ECG, and X-ray of thorax has been compared. Of these it has only been found possible to use the first three parameters for the graduated assessment of the effect of treatment.

Results

In the treated group the average time taken by the patients to regain a normal morning temperature was 5.1 days, in the control group it was 5 days. As can be seen from fig. 1 the charts of the average temperatures were as a whole *completely* alike in the two groups.

There was likewise no significant difference between the average daily amount of expectorate in the treated and control groups.

Table II shows the average *change* in the values found in the arterial blood in connection with the treatment instituted in *those patients in the group who had* obvious respiratory insufficiency (arterial oxygen saturation ≤ 80 and/or arterial pCO ≥ 45 *mm on first investigation).* No definite difference between the treated and control groups can be seen.

Discussion

As can be seen from table I the treated and control groups in the material described are completely comparable as to distribution of age and sex, and also as to the occurrence of chronic cor pulmonale and other relevant complications.

The average length of the history of bronchitis was also more or less similar in the two groups. In the majority of cases there was considerable chronicity in only 4 cases was the history shorter than 2 years The average length of history in both groups was over 10 years.

The material includes cases of all degrees of severity as the only criterion for inclusion has been the diagnosis of acute exacerbation in chronic bronchitis, defined as described above It is worth noting that nearly half the cases in each group could be described as severe, with obvious respiratory insufficiency which is apparent from the fact that in the treated group there were 16 episodes, and in the control group 14 episodes in which the arterial oxygen saturation was ≤ 80 % and/or $pCO_2 \geq 45$ mm at the first investigation. As is apparent from table II it was possible to obtain only a slight alteration in the arterial blood chemistry in connection with the therapy administered — another indication that from the respiratory point of view the acute exacerbation was only reversible to a lesser extent. The groups were also completely comparable in this respect.

It must be emphasized that none of the patients included in this material underwent tracheotomy During the period of the investigation very few patients who were admitted with an acute exacerbation of chronic bronchitis had such large amounts of secretion that tracheotomy was indicated. The condition of these patients was primarily characterised by severe obstruction of the respiratory tract, requiring immediate "mechanical relief, and schematic treatment following the rules indicated above was considered indefensible. For this reason these few patients having primary tracheotomy have not been included in the material.

The majority of patients in both treated and control groups received sodium benzyl penicillin (penicillin G) in doses of a million units, as during this period the use of this antibiotic was the standard therapy for acute exacerbations in chronic bronchitis in both the departments concerned in this study Only 3 episodes in each group were treated with tetracyclines. In 2 episodes in the treated and one in the control group penicillin was given together with another antibiotic. In only 2 cases (one in each group) was no antibiotic used In this respect also the groups are thus completely comparable.

The present account has demonstrated that the average course of temperature and amount of expectorate in patients with acute, febrile flare up of chronic bronchitis were the same whether or not the therapy included lung phynotherapy Two of the patients included had at least one episode in both treated and control groups, and in these patients there was no significant difference in the course of the disease during the various admissions.

Even if the cases with obvious respiratory insufficiency are considered alone it is not possible to demonstrate any difference, as the average increase in arterial oxygen saturation and reduction in arterial pCO were largely the same in the treated and control groups in association with the treatment instituted (table II)

If the groups in the present investigation are considered as a whole, it has thus not been possible to demonstrate any bene

ficial effect of daily lung physiotherapy on the course of acute febrile episodes in patients with chronic bronchitis. This does not however justify the conclusion that lung physiotherapy cannot be of value in individual cases with considerable amounts of bronchial secretions and the complications of these, e.g. large atelectases. We feel, however, that it is justified to conclude that at any rate the routine use of lung physiotherapy in acute febrile exacerbations of chronic bronchitis is not indicated.

Summary

During the period 1. I. 1961 to 1. IV. 1963 63 patients were admitted for treatment of a total of 68 acute episodes in the course of chronic bronchitis. With regard to the use of antibiotics, oxygen, digitalis, bronchiolytics, and bed rest etc. the treatment followed the same lines for all patients. On the other hand, daily lung physiotherapy ("expansion exercises", tapotement or vibrations and postural drainage) was given for 10 days in only 35 of the episodes, whilst this form for treatment was not used in the remaining 33 episodes.

The course of the illness in the two groups was assessed by daily measurement of temperature and amount of expectorate and by analysis of the arterial blood early and late in the period of treatment. The difference in the size of any lung infiltrates which might be present could, on the other hand, not be satisfactorily expressed quantitatively and has therefore not been used in the assessment.

Using the parameters mentioned it has not been found possible to discover any beneficial effect of daily lung physiotherapy on the course of acute exacerbations in chronic bronchitis. When given in the manner described, lung physiotherapy thus cannot be considered to be indicated as routine therapy for use in the acute flare-up of chronic bronchitis. This does not, however, exclude the possibility that the treatment can be of value in individual, selected cases, e.g. in the presence of large atelectases.

Acknowledgement

This study has been carried out with support from P. Carl Petersen's Fund, and King Christian X' Fund.

The authors thank Mrs. Bodil Thorup, physiotherapist, and Niels Jarlov, M. D., head of the Department of Physical Medicine, Glostrup Hospital, for their cooperation in the investigation reported.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

The Isotope Laboratory (Head: B. Chr. Christensen, M. D.) of the Medical Department (Head: A. Hecht Johansen, M. D.), The Finsen Institute, Copenhagen, Denmark

Chlornaphazine as a Bladder Carcinogen

By

Torben Thiede, Eva Chievitz and Børge Chr. Christensen

In the Medical Department of the Finsen Institute, Copenhagen, we have observed, during recent years, 7 cases of bladder tumours in patients with polycythaemia. The most important common feature of their histories appears to be the administration of chlornaphazine in large total doses.

In the course of the past 15 years numerous patients have been treated with chlornaphazine. In our department this drug has been used mainly for the treatment of polycythaemia. The therapeutic results in a series of 32 patients were reported in 1961 (6). A favourable effect was found in 75 . Dysuria was a side effect in 20 , and haematuria in a few. At the time of the analysis 7 of the patients had died. In one a carcinoma of the bladder was an accidental autopsy finding.

Analysing the causes of death in 250 polycythaemic patients from the whole of Denmark (7) we reported, in 1962, 3 cases of urinary-tract cancer among 26 persons treated with large doses of chlornaphazine, i.e. a daily dose of 300 mg or more for more than 3 months. Among the remaining 224 patients there was one case of hypernephroma, one of renal leiomyoma, and one of bladder papilloma. One patient had Boeck's sarcoid with infiltrations in the kidneys. In other words, there was a striking incidence of urinary-tract cancer among patients who had received large doses of chlornaphazine, so that ever since we have had our eye on this drug as a possible carcinogen.

Present series

Out of the polycythaemic patients followed in the Medical Department of the Finsen Institute a total of 61 have been treated with chlornaphazine at one time or other. The majority, however, have been treated periodically with P^{32} alternating with chlornaphazine, as this was expected to give rise to least side effects. Therefore, the total dose of chlornaphazine has usually been fairly small. Only 20 patients had received more than 100 g chlornaphazine, including 8 who had received 175 g or over. All 7 patients who developed tumour of the urinary bladder had received more than 100 g chlornaphazine.

Table I gives the data for the 7 patients with bladder tumours.

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Table I gives the data for the 7 patients with bladder tumours.

Table I Seven patients with bladder tumours

Case no.	1	2	3	4	5	6	7
Sex	♀	♀	♀	♂	♂	♂	♂
Age when tumour was diagnosed	71	54	67	64	61	65	62
Occupation	House-wife	House wife	House wife	Repre sentat	Joiner	Post master	Head of dept.
Type of tumour	Carcinoma	Carcinoma	Solid carc.	Solid carc.	Solid carc.	Papilloma	Papilloma
No. of yrs from polycythaemia was recognized until tumour was diagn.	10	20	12	6	11	9	9
Total dose of chlor naphazine in g (ca)	200	350	160	100	200	180	200
Duration of chlor naphazine therapy in yrs	4 1/6 Periodical	2 1/2 Uninter-rupted	11 Periodical	4 Periodical	2 Periodical	6 Periodical	5 Periodical
First sympt. or sign of tumour in time relation to chlor naphazine therapy	At time of discont.	During treatment	During treatment	At time of discont.	During treatment	At time of discont.	During treatment
First symptom or sign of tumour	Dysuria	Pollakiuria	Dysuria + pollakiuria	Gross haematuria	Gross haematuria	Micr haematuria	Gross haematuria
Other treatment	P^{32} 8 mC	X-ray irradiation of trunk. P^{32} 4 mC	None	P^{32} 4.5 mC	Chlorme thine P^{32} 16 mC	P^{32} 12 mC	P^{32} 15 mC
Smoking habits (daily consumption)	Unknown	Unknown	None	15—20 cigarettes	10—12 cigarettes	Moderate no cigarettes	Large for many yrs, later 10 cigarettes

It is evident that all 7 patients were over 50 years of age at the time when the bladder tumour was diagnosed. In other words, we are dealing with the age group in which cancer of the urinary bladder is most common, but 7 cases in 61 is considerably more than would be expected.

Polycythaemia does not predispose to cancer (10, 11). All 7 patients with tumours of the bladder had been suffering from polycythaemia for many years, 6 for about 10 years or longer. Our previous series of deceased polycythaemic patients (7) included 40 patients who had survived for more than 10 years after polycythaemia was diagnosed and who had not been treated with chlornaphazine. Autopsy was available for 15. None of these patients died of cancer of the urinary bladder or exhibited any signs of it. Thus, even at a late stage of the disease there does not seem to be a tendency to develop bladder tumours.

Reversely it may be ruled out that the condition should be interpretable as secondary polycythaemia due to the tumour. This possibility is definitely opposed by the long time interval between the diagnosis of polycythaemia and of the tumour. In addition, all the patients showed, apart from polyglobulism,

also changes in leukopoiesis and in platelet production, or splenomegaly indicating primary polycythaemia.

A common feature of the histories is that 6 of the patients had been treated with P[32] as well. However bladder tumours have never been reported as complication to radioactive therapy. Among the patients with polycythaemia in the Finsen Institute 46 have been treated with P[32] either alone or combined with other agents, without ever having received chlornaphazine. Among this group there is no known case of bladder tumour.

Occupationally the patients show no common features. Four were domiciled in Copenhagen and 3 in smaller towns. Lastly it may be mentioned that two were heavy cigarett smokers, two more moderate smokers, while one did not smoke at all. In two cases the smoking habits are unknown.

In all 7 cases chlornapharine had been administered in large quantities in the course of at least 2 years. In 6 instances there had been breaks in the treatment, while the 7th patient was treated without interruption for about 2½ years.

The first symptom or sign of the tumour was haematuria in 4 cases, dysuria or pollakiuria in 3. Example Case 3, 67-year-old housewife treated periodically through 11 years with chlornaphazine, total of about 160 g. One year after the treatment had been discontinued she was referred to the Finsen Institute for other treatment. She was complaining of headache, fatigue, and dizziness. Her appearance was polycythaemic, mucous membranes dark red, and the spleen enlarged. Hb 145 % = 21.3 g%. R.B.C. 6.7 mill./µl, W.B.C. 13,000 µl with few immature forms, platelet count normal. Haematocrit value 67 %, blood volume 81 ml/kg, cell volume 54 ml/kg. On admission she also reported periodical dysuria and pollakiuria with foul-smelling, cloudy urine for the past few years. Urine analysis revealed traces of protein. Microscopical examination 10–20 red cells and 5–10 white cells per field. Intravenous pyelography gave rise to suspicion of bladder tumour and cystoscopy + digital palpation revealed an inoperable tumour which had penetrated the base of the bladder.

As already mentioned, a total of 20 of our polycythaemic patients had received more than 100 g chlornapharine, and of this group 7 developed tumours of the bladder. Only 8 patients had received a total dose exceeding 175 g chlornaphazine and of them 5 developed bladder tumours. Among the 41 patients who had received less than 100 g chlornapharine there have been no signs of new growths in the urinary bladder.

Comments

Thus clinical experience definitely indicates that chlornaphazine is carcinogenic when used in large quantities for long periods of time. Theoretically this assumption is greatly supported by the knowledge regarding the effect of related substances. Chlornaphazine is dichlorodiethyl-β-naphthylamine. The first substance which was found to be capable of inducing cancer of the bladder in experimental animals was 2-naphthylamine (9). Since that time, numerous workers have reported the induction of bladder cancer in animals by oral administration of related substances (1, 2, 3). It is a characteristic finding that cancer usually develops at approximately the same time in animals of the same species who are exposed to ingestion of a given carcinogenic substance. The sequence of events is epithelial hyperplasia restricted to part of the bladder, precancerous changes, and lastly actual cancer. To explain why these neoplasms appear in the bladder and not in other organs, Boyland (4) has reported that the actual carcinogens are orthoaminophenols. Thus, in the liver 2-naphthylamine becomes 2-amino-1-naphthol. However already in the liver detoxication takes place by the formation of compounds with glucuronides, sulphates, and phosphate esters, and in this form the substances are excreted in the bile and urine. This explains why carcinoma does not develop in the liver since in this organ the concentration of carcino-

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genic agents will always be very low. In other parts of the body the substance is present in a bound non-carcinogenic form. The urine, however, always contains β-glucuronidases which liberate the actual carcinogen. Moreover it may be mentioned that chemically chlornaphazine is very similar to some of the substances which have induced the largest number of cases of bladder carcinoma among workers in the dye industry, in particular β-naphthylamine. Ferguson et al. (8) in 1934 reported 23 cases of bladder carcinoma in workers exposed to β-naphthylamine in their daily work. Vigliani et al. (12) described 3 cases of bladder carcinoma and 6 of papilloma among 26 workers in a factory making β-naphthylamine. In most countries β-naphthylamine is no longer produced or else the production is restricted to a very few plants. Case et al (5) have pointed out that the risk of developing cancer of the bladder run by workers in the β naphthylamine industry is 61 times that in the general population.

Thus, clinical experience of chlornaphazine and experimental experience of substances related to chlornaphazine strongly suggest that chlornaphazine used in large quantities through a long period of time, is a carcinogen whose target is the urinary bladder. As already mentioned, cancer of the bladder did not occur among the 41 patients who were treated with chlornaphazine in total doses lower than 100 g. In this connection it may be pointed out that we aim at regular follow up with frequent urine analysis and in the event of the slightest suspicion of tumour immediate intravenous pyelography and cystoscopy.

From table I it may be seen that all the cases of tumour were observed in direct relation to chlornaphazine therapy. In accordance with the animal experiments in which the cancer usually develops a given time after the exposure to the carcinogenic agent, development of cancer in the urinary bladder of polycythaemic patients should not be expected to occur years after the withdrawal of chlornaphazine given in fairly small doses. On the basis of the present small series it is impossible to state any absolute value for the dose of chlornaphazine which may be given to man before the carcinogenic effect manifests itself, but it seems to be above 100 g in all cases. Moreover the chlornaphazine therapy apparently has to be administred for several years before development of cancer occurs.

It is evident from the analysis of the causes of death in a series of 250 patients with polycythaemia (7) that the disease definitely requires treatment, that venesection as the only therapeutic method is absolutely insufficient, and that treatment with heavy ionizing irradiation often induces leukaemia. According to the present investigation, long term therapy with chlornaphazine involves a risk of bladder carcinoma. As yet, our knowledge concerning the possible side effects of other mitostatics is insufficient.

Accordingly, chlornaphazine should not be used for long term therapy and never in the first attempt at controlling polycythaemia. It should be reserved for those cases in which remission cannot be brought about by other mitostatics or P^{32}. During and after the treatment the patients should be kept under supervision.

Summary

Among the polycythaemic patients followed in the Medical Department of the Finsen Institute, Copenhagen, 61 have been treated with chlornaphazine, including 20 who have received a total dose exceeding 100 g. Among this latter group

7 cases of bladder tumour have occurred. Of 8 patients who had received 175 g chlornaphazine or more, 5 developed bladder tumour. The similarity of chlornaphazine and the substances which have induced bladder tumour in industrial workers is pointed out. It is concluded that chlornaphazine, administered in large doses through long periods of time, is carcinogenic and has the urinary bladder as its primary target organ. Chlornaphazine is inadvisable, if other treatment can bring about a remission.

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genic agents will always be very low. In other parts of the body the substance is present in a bound non-carcinogenic form. The urine, however, always contains β-glucuronidases which liberate the actual carcinogen. Moreover, it may be mentioned that chemically chlornaphazine is very similar to some of the substances which have induced the largest number of cases of bladder carcinoma among workers in the dye industry, in particular β-naphthylamine. Ferguson et al. (8) in 1934 reported 23 cases of bladder carcinoma in workers exposed to β-naphthylamine in their daily work. Vigliani et al. (12) described 3 cases of bladder carcinoma and 6 of papilloma among 26 workers in a factory making β-naphthylamine. In most countries β-naphthylamine is no longer produced or else the production is restricted to a very few plants. Case et al. (5) have pointed out that the risk of developing cancer of the bladder run by workers in the β-naphthylamine industry is 61 times that in the general population.

Thus clinical experience of chlornaphazine and experimental experience of substances related to chlornaphazine strongly suggest that chlornaphazine, used in large quantities through a long period of time, is a carcinogen whose target is the urinary bladder. As already mentioned, cancer of the bladder did not occur among the 41 patients who were treated with chlornaphazine in total doses lower than 100 g. In this connection it may be pointed out that we aim at regular follow-up with frequent urine analysis, and in the event of the slightest suspicion of tumour immediate intravenous pyelography and cystoscopy.

From table I it may be seen that all the cases of tumour were observed in direct relation to chlornaphazine therapy. In accordance with the animal experiments, in which the cancer usually develops a given time after the exposure to the carcinogenic agent, development of cancer in the urinary bladder of polycythaemic patients should not be expected to occur years after the withdrawal of chlornaphazine given in fairly small doses. On the basis of the present small series it is impossible to state any absolute value for the dose of chlornaphazine which may be given to man before the carcinogenic effect manifests itself, but it seems to be above 100 g in all cases. Moreover, the chlornaphazine therapy apparently has to be administred for several years before development of cancer occurs.

It is evident from the analysis of the causes of death in a series of 250 patients with polycythaemia (7) that the disease definitely requires treatment, that venesection as the only therapeutic method is absolutely insufficient, and that treatment with heavy ionizing irradiation often induces leukaemia. According to the present investigation, long-term therapy with chlornaphazine involves a risk of bladder carcinoma. As yet our knowledge concerning the possible side effects of other mitostatics is insufficient.

Accordingly, chlornaphazine should not be used for long term therapy and never in the first attempt at controlling polycythaemia. It should be reserved for those cases in which remission cannot be brought about by other mitostatics or P[32]. During and after the treatment the patients should be kept under supervision.

Summary

Among the polycythaemic patients followed in the Medical Department of the Finsen Institute, Copenhagen, 61 have been treated with chlornaphazine including 20 who have received a total dose exceeding 100 g. Among this latter group

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

The Department of Cardiology and the Department of Clinical Physiology
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By

B. Christensson, A. Gustafson and H. Westling

The diagnosis of coronary insufficiency is sometimes based upon electrocardiographic changes, either spontaneously occurring during an attack of chest pain, or seen after provocation, usually in the form of a standardized exercise test, e. g. Master (10) Sjöstrand (12). The electrocardiographic pattern of "flatly depressed and sagging" ST-segments is considered typical for coronary insufficiency e. g. Mattingly (11) and develops during the exercise. The electrocardiographic changes usually disappear in a few minutes after stopping the work test; sometimes the terminal part of the ST-segment remains depressed longer so that a biphasic T-wave with normal initial ST segment may be seen at 2—6 min. after the exercise. In other cases the ST-depression vanishes more rapidly without any isolated T-wave changes.

It is well known that treatment with digitalis glucosides may cause similar changes in the exercise electrocardiogram also in patients, whose electrocardiograms at rest are not much influenced by digitalis. Apparently the same holds for quinidine, e. g. Dumond (1).

Abnormalities in the electrolyte balance may also cause electrocardiographic changes. Thus a lowered serum potassium level, e. g. during treatment with saluretics, is often associated with slight ST segment depression, diminution of the T-wave and the appearance of a positive after-potential in diastole. Previous observations (1, 3) and personal experience indicate that such changes may cause an erroneous diagnosis of coronary insufficiency. However, most of the observations mentioned were made on patients with arterial hypertension and it was thus not possible to exclude abnormalities in myocardial blood flow or ventricular hypertrophy as contributory causes of the electrocardiographic changes.

In the present study diuretics were given to young normal subjects in a dosage such that changes in the serum electrolyte pattern were provoked. The

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Fig 1 Pulse rates at rest and during different work loads before, during and after administration of chlorthalidone, 100 mg daily Mean values in 3 persons.

Fig 2 Pulse rates at rest and during different work loads before during and after administration of chlorthalidone, 200 mg daily Mean values and S. E. of means in 6 persons.

electrocardiographic changes in connexion with exercise were studied. In addition measurements of the pulse rate and blood pressure were made

Material and methods

Observations were made on 13 healthy subjects, mostly students and firemen. Some subjects were active sportsmen, but the majority were of average physical condition.

ECGs were recorded at rest, using the following leads I, II III aVR, aVL, aVF and chest leads from the conventional electrode positions 1 2 4 5, 6 and 7 The indifferent electrode for chest leads was V In addition chest leads at rest were taken using the right arm as indifferent electrode (CR leads) During exercise chest leads were taken from positions 2, 4 5 and 7 with the indifferent electrode on the forehead (CH leads)

The subjects were studied before during and after an exercise test on a bicycle ergometer (4) using successively increasing loads according to the principles described by Sjostrand (12) Pulse rates and ECG were recorded every second minute until a steady state on a particular work load had been reached after which the load was increased. Steady state was said to exist when the difference in pulse rate between two observations was smaller than 5 beats/min. Systolic blood pressure was measured by the auscultatory indirect method. Measurements during exercise were made with the subject's arm relaxed. Diastolic pressure measurements were sometimes difficult to obtain during exercise and these observations were therefore not analysed The reliability of auscultatory pressure measurements during exercise has been studied by Karlefors and Westling (6)

6–8 exercise tests were made on each subject, the first one or two being preliminary observations to make the subject familiar with the procedures. Exercise tests were then performed during drug treatment, and some days after stopping the treatment and normalization of electrolyte and fluid balance.

Venous blood samples were taken with slight stasis avoiding muscular contractions of the arm. Sodium, potassium and chloride concentration were determined in serum and hemoglobin concentration and hematocrit value in whole venous blood.

The following drugs were given

Chlorthalidone (Hygroton" Geigy) dosage 100 mg (3 subjects) or 200 mg each day (6 subjects)

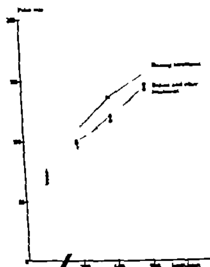


Fig. 3. Relation between pulse rate and work load before, during and after administration of chlorthalidone, 200 mg daily. Mean [v]alues in 6 persons. ● before treatment, ○ during treatment, × after treatment. Time intervals in relation to treatment, see fig. 2.

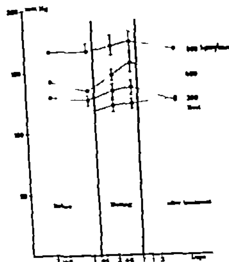


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Hydrochlorothiazide (Esidrex Ciba) dosage 25 mg three times daily (4 subjects).

Some subjects received 0,25 g acetylsalicylic acid daily as "placebo" treatment.

Results

1. *Weight loss, hemoconcentration and electrolyte changes*

A loss in body weight and some hemoconcentration were regularly seen during treatment with diuretics. Using the 100 mg dose of chlorthalidone or hydrochlorothiazide 25 mg three times daily the dehydration was only moderate but with 200 mg chlorthalidone per day the body weight declined by [a]verage of 2.2 kg. The hematocrit value rose from 41 to 47 [%] in 6 days. This indicates a loss in plasma volume of more than 1/2 l and a proportional loss of extracellular fluid.

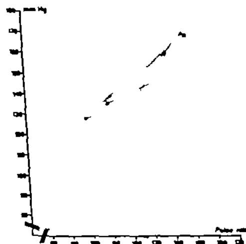


Fig. 5. Relation between blood pressure and pulse rate on different work loads (300, 600 and 900 kpm/min.) during administration of chlorthalidone 200 mg daily. Mean values in 6 persons. Filled symbols: before and after treatment. Open symbols: during treatment. For time intervals in relation to treatment see figs. 2 and 4.

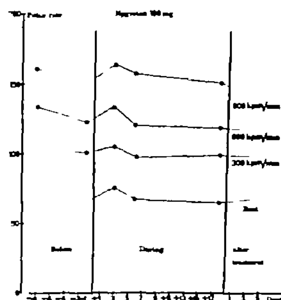


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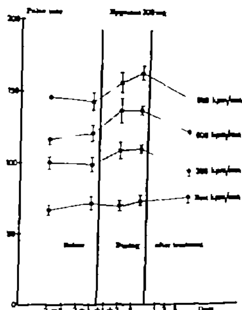


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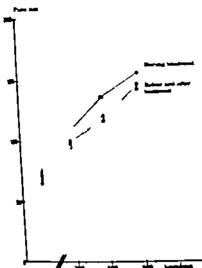


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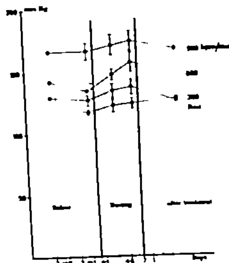


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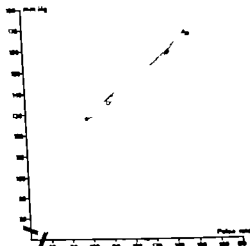


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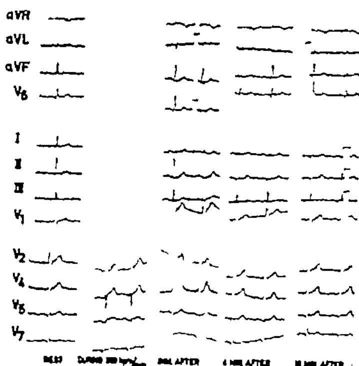


Fig 6. Exercise ECG in a 34-year-old healthy male before administration of chlorthalidone. Na 153, K 4.0 Cl 101 mEq/l

Both drugs decreased sodium and chloride levels in the serum. With hydrochlorothiazide the changes were not significant. Chlorthalidone had similar effects in the two dosages given the sodium level decreasing from 146 to about 140 mEq/l and chloride from 104 to about 95 mEq/l after 6—8 days of treatment.

The average initial value for serum potassium was 4 3 mEq/l After 6 days of hydrochlorothiazide an average value of 3 5 mEq/l was noted (4 cases) Chlorthalidone, 100 mg daily in 3 cases, lowered the values to 3 3 and 3 0 mEq/l after 4 and 8 days administration respectively Then a restitution seemed to take place, the mean value after 17 days being 3 5 mEq/l. Chlorthalidone, 200 mg daily in 6 cases, decreased serum potassium to average values of 3 5 and 2 9 mEq/l after 3 and 6 days respectively

2 *Pulse rate and blood pressure during exercise*

The present investigation was primarily planned as a study of electrocardiographic changes during exercise in normal subjects treated with saluretics but the changes in pulse rate and blood pressure appear to be of sufficient interest to merit a brief description.

During diuretic treatment a rise in pulse rate during exercise was seen. This effect vanished during continued treatment with chlorthalidone in moderate dosage (100 mg/day fig 1) but with high dosage (200 mg/day fig 2) it persisted until the treatment had to be stopped because of tiredness and other subjective sensations in the subjects. The linear relation between pulse rate and work load persisted during treatment (fig 3) The higher pulse rate during treatment corresponded to a 20—25 % decline in the physical working capacity (12) The higher pulse rate is likely to be due to a diminished stroke volume as a consequence of the acute dehydration and hemoconcentration (section 1 above) Other factors such as changes in myocardial excitability and contractility may of course also play a role.

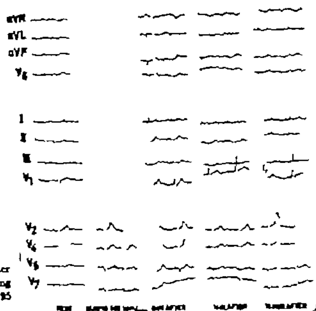


Fig. 7. Same person as in fig. 6 after administration of chlorthalidone 200 mg daily for 3 days. Na 140, K 3.5; Cl 95 mEq/l.

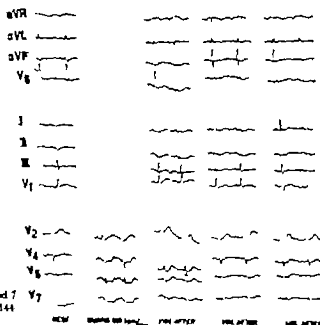


Fig. 8. Same person as in fig. 6 and 7 after 5 days of treatment. Na 144 K 2.3, Cl 104 mEq/l.

The systolic pressure regularly increased during exercise. F en during max mal treatment with saluretics there was no tendency to a diminished blood pressure rise during exercise on the contrary the systolic blood pressure increased during treatment (fig. 4). This may be due to the fact that the relative work load

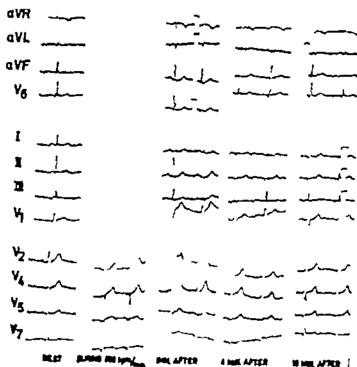


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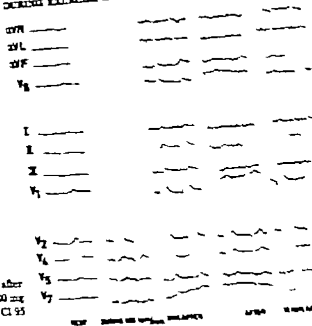


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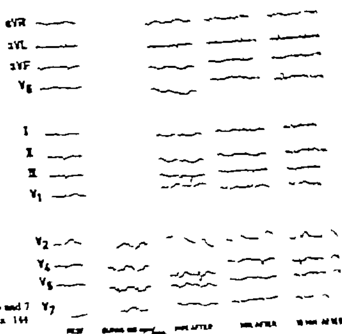


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The systolic pressure regularly increased during exercise. Even during maximal treatment with saluretics there was no tendency to a diminished blood pressure rise during exercise; on the contrary the systolic blood pressure increased during treatment (fig. 4). This may be [illegible] [illegible] has the relative work load

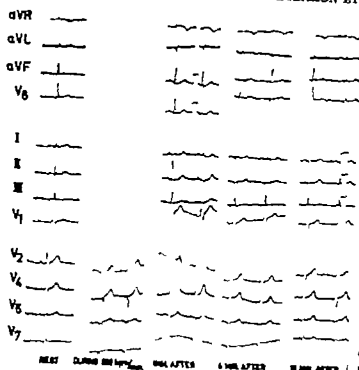


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exercise (fig. 6) During treatment with diuretics a flattening of the T wave and a positive diastolic after potential appeared in the electrocardiogram at rest, (figs. 7 and 8) Significant ST-depressions were not seen at rest. In about half of the subjects abnormal electrocardiographic changes during exercise were observed this occurred in cases with a serum potassium level around 3 mEq/l or less. The ST-segments were depressed during exercise and the T wave often inverted (fig. 8) Such changes were often seen also at low work loads, and even with quiet sitting on the bicycle. The changes disappeared rapidly after exercise and were often already absent in the first tracing, which was recorded about 1/2 min. after the exercise. In no case were changes present 4 min. after exercise.

The similarity between the exercise electrocardiogram in coronary insufficiency and that during treatment with diuretics will be apparent from a comparison of figs. 8 and 9 Fig. 10 shows more advanced electrocardiographic changes in a case of coronary insufficiency such changes were never seen during treatment with diuretics.

Electrocardiographic changes were seen with both diuretics, but were more frequent and pronounced with chlorthalidone in the higher dosage. This is in conformity with the observations on the pulse rate and electrolyte balance.

Discussion

The administration of saluretic drugs to normal subjects leads essentially to the same changes as in patients with edema or hypertension (8, 9) i.e. a loss of body water and electrolytes. Hydrochlorothiazide and chlorthalidone had effects similar in nature but in the dosage used the effect of the former drug was not very marked. In conformity with previous results, e.g. Varnauskas et al. (13) and Wilson & Freis (15) we obtained evidence for a reduced blood volume which was the probable explanation for an increased pulse rate during exercise. It seems reasonable to assume that this was caused by a lowered stroke volume. On the lower dose of chlorthalidone an adaptation appeared to take place this is also in accordance with current views on the mode of action of saluretics. The initial reduction in blood pressure is thought to be due to a reduced blood volume but other mechanisms may later come into play (for discussion see Wilson & Freis (15) Winer (16) Varnauskas et al (13))

Indirect estimations of the systolic blood pressure during exercise are of course not so accurate, but still fairly well correlated to direct measurements (6) and the present observations may therefore be of some significance. The lack of effect of even an intensive diuretic treatment upon the blood-pressure rise during exercise was a rather unexpected finding Dunér (2) has shown the effectiveness of chlorthalidone in reducing the rise in blood pressure that occurs in hypertensive patients during exercise. The treatment given to the present series of normal subjects would surely have caused a reduction in blood pressure in hypertensive subjects.

The present observations show that an exercise test may augment ST and T changes induced by treatment with saluretic agents the electrocardiographic response during exercise could be misinterpreted as a sign of coronary insufficiency However the changes in ST and T caused by saluretics had their maximum during exercise and disappeared quickly afterwards. In the material significant

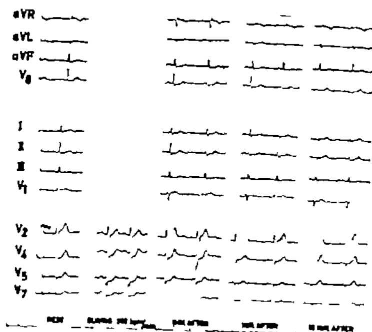


Fig 9. Exercise ECG in a 48-year-old male with angina pectoris and pathological coronary arteriogram. Note ST-depression during exercise with rapid disappearance afterwards.

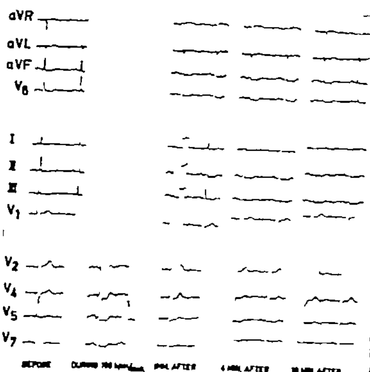


Fig 10 Exercise ECG in 64-year-old male with angina pectoris and pathological coronary arteriogram. Note biphasic – negative T wave 1 min. after exercise

was higher during treatment thus the blood pressure during exercise in relation to the pulse rate was essentially unchanged (fig 5)

The administration of hydrochlorothiazide did not lead to any significant changes in the pulse rate and blood pressure reaction during exercise

3 *Electrocardiographic changes*

All subjects had normal electrocardiograms at rest and during and after severe

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

The Department of Pediatrics, Kronprinsessan Lovisas Barnsjukhus and
the Department of Clinical Physiology Serafimerlasarettet, Karolinska Institutet,
Stockholm, Sweden

Blood Flow in the Extremities at Rest, During Reactive Hyperaemia and after Ganglionic Block in Cases of Friedreich's Ataxia

By

Claes Thorén and John Wahren

Among the hereditary ataxias, Friedreich's ataxia stands out as a comparatively well defined disease (6) It usually appears first at school age and progresses relatively slowly with increasing unsteadiness of gait owing to spinal ataxia and weakness of the legs. After about 10 years the ability to walk is usually lost, owing mainly to pareses of the legs.

It has long been known that Friedreich ataxia is often accompanied by a myocardial disease, to which increased interest has been devoted in recent years (1 10, 11 15) ECG changes in the form of arrhythmias and general T-wave inversion often appear The pathological anatomical picture is characterized by general myocardiac degeneration with fibrotic replacement and subintimal proliferation of coronary arteries (13)

A systolic murmur is commonly found over the base of the heart. Ikkos and Thorén (12) however were unable, in heart catheterization study to demonstrate any organic cause for this murmur Only in some cases there was a large cardiac output and the a—v O difference was relatively small in relation to the oxygen uptake. The blood flow in the extremities, as determined by occlusion plethysmography was in these cases moderately increased. On the other hand, cold and cyanotic feet, suggesting reduced peripheral circulation are wellknown and frequent phenomena in the more advanced and crippling stages of Friedreich's ataxia.

In view of the suspicion that a coronary arterial disease is present in Friedreich's ataxia (13 14 21) the present study was undertaken to determine what disorders, if any are present in the peripheral circulation in patients with varying degrees of this disease

Material

The material consists of 29 cases with the typical syndrome of Friedreich ataxia, all previously described elsewhere (23) For practical reasons the same case numbers have

Submitted for publication November 27 1963.

electrocardiographic changes during exercise occurred only at low serum potassium levels (about 3.0 mEq/l or less). For practical purposes it is therefore recommended to analyse the serum electrolytes in those cases with a pathological exercise electrocardiogram in which there is suspicion of electrolyte changes, e.g. due to diuretic treatment. Special care should also be taken in the interpretation of exercise electrocardiogram in cases with a resting electrocardiogram with a flat T wave and a diastolic afterpotential.

An attempt was made to analyse more precisely the role of the potassium ion in the electrocardiographic changes observed. However successful substitution of the potassium "deficit" could not be carried out without simultaneous changes in sodium and chloride concentrations. Nevertheless, on the basis of previous evidence (7–14) it would appear likely that the potassium ion is the most important one.

Summary

The changes in pulse rate, systolic blood pressure and electrocardiogram during exercise were studied before, during and after treatment of normal subjects with chlorthalidone or hydrochlorothiazide.

An increased pulse rate during exercise was seen during intensive treatment with chlorthalidone. The systolic blood pressure during exercise was not decreased.

The drugs induced moderate electrocardiographic changes at rest. During exercise ST- and T-changes often occurred, which could be misinterpreted as signs of coronary insufficiency. Electrocardiographic changes during exercise may be caused by electrolyte disturbances, notably hypokalemia.

Acknowledgement

The present study was aided by a grant from the Swedish National Association against Heart and Chest Diseases.

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*Table II. Individual values for sex, age, duration of disease, segmental volume, pulse rate, forearm and calf blood flow at rest and during reactive hyperaemia (R.H.) before and after ganglionic blocking in the different groups. Values indicated by * were obtained after lumbar ganglionic blocking*

Case no. Sex	Age (yrs)	Duration of disease (yrs)	Segmental volume		Pulse rate	Rest	R.H.	Rest	R.H.	Ganglionic blocking				
						Forearm		Calf		Pulse rate	Rest	R.H.	Rest	R.H.
			Fore arm (ml)	Calf (ml)		(ml/min/100 ml)					Forearm		Calf	
											(ml/min/100 ml)			
Group I														
6 b ♂	14	5	140	220	98	3.1	45.0	2.2	34.0	—	—	—	—	—
7 ♂	16	7	239	346	80	2.2	20.1	2.4	20.4	88	2.4	50.9	4.5	27.0
14 b ♂	12	5	193	335	83	3.7	23.9	2.7	14.1	88	4.5	50.4	4.3	15.2
17 o	16	3	167	383	98	8.7	45.0	7.2	30.0	—	—	—	—	—
19 ♀	16	7	163	329	98	2.2	38.4	1.8	20.4	105	1.4	42.0	3.9	24.3
32 b ♀	12	7	122	229	80	6.2	32.0	2.2	16.0	—	—	—	—	—
Group II														
6 ♂	17	6	202	280	94	5.5	42.0	3.0	18.0	—	—	—	—	—
10 ♀	13	10	148	280	88	4.1	—	3.5	—	—	—	—	—	—
11 b ♀	14	4	156	301	112	3.8	37.0	4.7	24.8	120	4.3	36.3	5.8	28.6
14 ♂	14	7	164	298	98	2.7	20.1	0.8	6.1	98	3.0	34.1	4.2	7 1
19 b ♀	18	11	171	329	80	0.7	16.8	2.8	14.9	—	—	—	—	—
23 o	22	15	299	383	68	2.1	21.3	2.2	23.1	—	—	—	—	—
33 ♂	14	12	211	378	100	0.7	31.6	3.6	32.6	115	2.4	21 4	6.7	26.1
35 b ♂	14	12	207	338	100	6.6	19.7	3.7	13 4	96	2.5	19.2	7.7	32.2
Group III														
4 ♂	15	9	125	202	110	4.2	30.1	0.9	10.1	—	—	—	—	—
9 ♀	22	15	144	296	110	3.6	28.0	2.8	16.0	—	—	—	—	—
11 ♂	22	16	219	350	88	2.1	24.2	1.0	15.2	80	1.5	24.8	4.4	28.0
26 b ♀	43	23	202	312	98	<0.5	7.8	0.7	5.1	96	1 7	13.8	1.5	10.9
31 ♀	26	12	215	425	65	3.2	21.2	1.9	14.8	—	—	—	—	—
33 b ♂	29	18	264	370	74	2.2	23.5	0.7	15.9	88	1.5	23.1	2.7	18.4
Group IV														
2 ♂	26	24	211	338	68	2 7	—	1.2	—	—	—	—	—	—
3 b ♀	14	11	181	249	98	3.0	15.1	<0.5	3.7	102	2.2	15.6	1.2	7.2
5 b ♂	23	17	290	335	70	2.5	18.6	1.6	7.8	74	2 9	26.1	5.4	17.3
8 ♂	21	18	211	249	84	3.1	28.9	0 8	17 4	—	—	—	2.3*	21 4
19 ♀	25	18	171	244	68	<0.5	20 7	0.9	8.9	89	1.6	8.8	5.4	23.7
27 ♂	23	16	212	229	72	1 1	18.7	1 4	12.8	—	—	—	4.6*	21.2*
28 ♀	16	11	180	323	78	5 0	40	2.4	10.0	—	—	—	—	—
30	43	35	193	329	79	1.5	21	<0.5	4.5	—	—	—	—	—
32 ♂	22	10	144	176	100	3.6	—	2.5	—	—	—	—	—	—

Table I. Case material showing age, duration of disease and body weight (mean, SE of mean, and SD)

Group	No. of cases	Age	Duration of disease	Weight
I	6	14.3	5.7	45.5
		0.8	0.7	2.6
		2.0	1.6	6.5
II	8	15.8	9.6	46.8
		1.1	1.3	3.1
		3.1	3.7	8.8
III	6	26.5	15.5	50.3
		4.2	2.0	5.1
		10.2	4.9	12.5
IV	9	23.7	17.8	48.4
		2.8	2.6	3.7
		8.5	7.8	11.2

been used in the present paper, each sibship has a number, the individual siblings being differentiated by small letters.

Age, duration of disease and weight are given in table I. The material has been grouped according to physical handicap, i. e. the degree of functional impairment. Group I = slight physical handicap, i. e. slightly unsteady gait, though able to run. II = moderate handicap, i. e. able to walk without support but unable to run. III = severe handicap, i. e. unable to walk without support, wheel chair mostly required. IV = very severe handicap, wheelchair definitely required. The breakdown of the material by degree of handicap is given in table I.

All cases but one (11 b) showed pathological ECG's suggestive of cardiomyopathy (23). In 14 cases right heart catheterization was performed. Four cases had diabetes mellitus, two with durations of 22 (case 3 b) and 5 (case 30) years respectively. In the other two cases the disease was detected in the course of the clinical examination. Two other patients had a pathological glucose tolerance, possibly indicating a prediabetic status. None of these patients showed signs of diabetic angiopathy as judged from examination of the retinal vessels, radiography of the legs or endogenous creatinine clearance.

A group of 14 normal subjects, group N, consisting of 5 healthy students, 3 males and 2 females, mean age 24.0 years (range 23–25) and 9 healthy schoolchildren, 6 boys and 3 girls, mean age 12.1 years (range 11–13) was also examined.

Methods

Venous occlusion plethysmography. The blood flow in the extremities was measured at rest and after 5 min. arterial occlusion with venous plethysmography according to Dohn (2) modified by Graf and Westersten (7) using a 5 cm broad volume-recording cuff. The segment volume was calculated after measuring the circumference with a millimetre tape and approximating to a cylinder. Determining the blood flow at rest in the calf, Strandell and Wahren (22) found with this method a methodological error expressed as coefficient of variation of 15.2 % for an individual observation based on 10 consecutive inflow curves. The apparatus and technique used were the same as in the present study. Blood flow values of less than 0.5 ml/min./100 ml are given in table II as < 0.5 and treated in the statistical processing as 0.5.

The error of the method expressed as coefficient of variation in measurements of the size of the reactive hyperaemia 10 sec. after arterial occlusion was 7.5 %. This value is based on double determination at 15 minute intervals in healthy normal persons (n = 10). Pulse rate was calculated from the pulsations in the plethysmogram.

Oscillography. Arterial pulsations in calves and forearms were recorded with 5 cm broad rubber cuffs and Infraton-Puls oscillograph OS 3, B. Boucke AB, Tubingen, West Germany. Maximum pulse amplitude was measured with a ruler and is expressed, after volume calibration, in cm³.

Digital plethysmography. Volume pulsations from toes were recorded with a piezo-electric crystal. This was in air communication with an airtight chamber enclosing the toe. The signal from the pressure receptor was simultaneously recorded with an ECG on a direct writing ink jet recorder. The inclination time of the pulsations was calculated ad modum Lund (18), taking the mean value for three pulsations.

Table III. Forearm and calf blood flow (ml/min/100 ml) at rest and 10 sec after release of 5 min. arterial occlusion before and after ganglionic blocking in the different groups. Mean, SE of mean, SD and no. of observations

	Before ganglionic blocking			After ganglionic blocking		
	N	I + II	III + IV	N	I + II	III + IV
Forearm at rest	4.77 0.60 1.89 10	3.72 0.62 2.30 14	2.59 0.34 1.30 15	2.44 0.47 1.04 5	3.21 0.52 1.36 7	1.90 0.23 0.56 6
Forearm reactive hyperaemia	40.7 3.6 11.3 10	31.0 2.8 10.0 13	22.6 2.3 8.3 13	42.8 2.3 5.2 5	30.6 3.0 8.1 7	18.7 2.8 7.0 6
Calf at rest	4.02 0.47 1.48 10	3.04 0.41 1.53 14	1.31 0.19 0.75 15	5.34 0.26 0.57 5	5.30 0.55 1.46 7	3.44 0.61 1.72 8
Calf reactive hyperaemia	35.5 1.7 5.3 10	20.8 2.2 7.0 13	10.9 1.3 4.8 13	36.8 1.3 2.9 5	22.9 3.3 8.7 7	18.5 2.4 6.8 8

Table IV. Differences in blood flow values between the various groups and within the groups after ganglionic blocking. t-values and probability (p) that the difference is caused by random factors

	N — (I + II)	N — (III + IV)	(I + II) — (III + IV)	After ganglionic blocking		
				N	(I + II)	(III + IV)
Forearm at rest	1.18 $p < 0.4$	3.18* $p < 0.01$	1.62 $p < 0.1$	2.72 $p < 0.1$	0.11 $0.8 < p$	0.27 $p < 0.8$
Forearm reactiv hyperaemia	2.18* $p < 0.05$	4.21 ** $p < 0.001$	2.33 $p < 0.05$	2.35 $p < 0.1$	0.53 $p < 0.6$	0.14 $0.8 < p$
Calf at rest	1.55 $p < 0.2$	6.06** $p < 0.001$	3.87*** $p < 0.001$	7.80** $p < 0.005$	6.20** $p < 0.001$	4.91** $p < 0.002$
Calf reactiv hyperaemia	5.00* $p < 0.001$	11.64** $p < 0.001$	3.79* $p < 0.001$	1.56 $p < 0.2$	1.43 $p < 0.3$	4.81** $p < 0.002$

are given in fig. 2 and table III. Reactive hyperaemia in the forearm was significantly reduced ($p < 0.01$) in group III + IV. Here too, however, the calf blood flow changes are the most pronounced, and the blood flow measured declines with increasing invalidity. Significant differences exist in respect of the extent of reactive hyperaemia in the calf between groups I + II and III + IV and

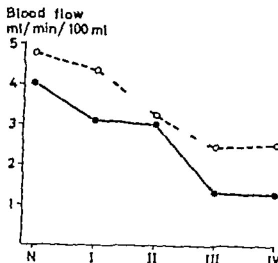


Fig 1 Resting blood flow in forearm (○---○) and calf (●——●) in a group of 10 healthy subjects (N) and in groups of Friedreich cases with increasing degree of physical handicap (I—IV)

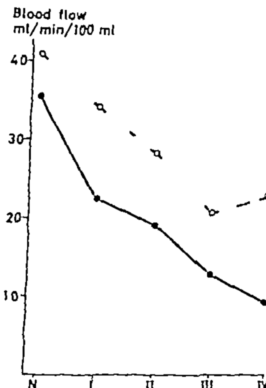


Fig 2. Blood flow in forearm and calf 10 sec after release of arterial occlusion. Symbols as in fig. 1

Temperature measurements Skin temperature on the sole of the foot, dig I and dig. III was measured with a thermocouple connected to a direct reading mirror galvanometer (TE 3 Ellab, Denmark) The mean value of the three readings was calculated. The measurements were made after heating for 30 min. with a heat cupboard placed over the thorax.

Ganglionic block. General ganglionic block was effected by the intramuscular administration of chlorisondamine dimenthochloride (Ecolid®) The dose used was 0 1 mg per kg body weight. The patient rested for an hour after the administration of Ecolid before measurements were carried out.

In three cases, staff from the Department of Anaestesiology performed lumbar sympathetic block with 30 ml 0.5 % Carbocain® injected at the level of the second and fourth lumbar vertebrae on the venterolateral side In all cases an extinguished sudomotor reaction following pain stimulation could be demonstrated in skin resistance measurements, indicating effective block (16)

Results

1 Blood flow

Rest. The individual values are given in table II The blood flow in forearms and calves at rest in the different groups is illustrated in fig 1 In the calf in particular the blood flow at rest declined with increasing invalidity Analysis of variance, however showed that the main inter group difference is between groups N I + II and III + IV and consequently the material has been combined in this way (table III) Significantly lower blood flow in the forearm at rest was measured in group III + IV as compared with the normal group ($p < 0.01$) Significantly impaired calf blood flow was recorded in group III + IV as compared with group N and group I + II ($p < 0.001$) (table IV) The mean pulse rate at rest for groups I—IV was 87.5 ± 14.4 beats/min (mean ± S D) In group N the pulse rate was 73.2 ± 9.7 which is probably significantly lower ($p < 0.05$)

Reactive hyperaemia Forearm and calf blood flow in the different groups 10 seconds after 5 minutes arterial occlusion

Table VI. Maximal amplitude of pulsations at oscillography (cm), inclination time in digital plethysmogram (csec), skin temperature (°C) and segment volume (ml) in 8 Friedreich cases from groups III and IV and in a group of 6 healthy children (age 11—13 years). Mean, SE of mean, SD, no. of observations, t-values and probability (p) that the difference between the two groups is caused by random factors

Pulsations						Inclination time		Skin temperature		Segment volume			
Group F			Group N			F	N	F	N	Group F		Group N	
Arm	Calf	Calf/Arm	Arm	Calf	Calf/Arm					Arm	Calf	Arm	Calf
0.17	0.18	1.06	0.15	0.25	1.68	10.2	10.3	25.0	33.7	189	272	172	338
0.01	0.01	0.05	0.01	0.01	0.04	0.2	0.3	2.0	0.2	16	20	6	10
0.04	0.05	0.22	0.02	0.03	0.15	0.5	1.0	5.2	0.8	46	56	15	24
16	16	16	12	12	12	11	12	7	12	8	8	6	6
2.00	4.[illegible]6***	8.57***	—	—	—	0.15	—	5.81	—	1.43	2.71	—	—
$p<0.1$	$p<0.001$	$p<0.001$	—	—	—	$p<0.9$	—	$p<0.001$	—	$p<0.2$	$p<0.05$	—	—

degree of atrophy has been taken as the difference between the calculated and the measured volume, expressed as a percentage of the calculated volume. In group I + II the measured arm segment volume was 12.1 ± 15.1 % lower than the calculated ($p < 0.001$), the leg volume 24.5 ± 12.0 % less ($p < 0.001$). The corresponding figures for group III + IV were 14.3 ± 17.0 % ($p < 0.01$) and 36.2 ± 15.3 % ($p < 0.001$).

Discussion

The investigation has demonstrated reduced peripheral blood flow in patients with Friedreich ataxia. The disorder seems to effect both the blood flow at rest and the extent of the reactive hyperaemia. The changes are of a progressive nature and are most pronounced in the lower extremities. The picture of the disease presents a number of factors of potential importance for the reduced blood flow in the extremities. The two most important would seem to be the inactivity and the cardiac disease.

Atrophy

The reduction in blood flow appears to grow with the duration of the disease. This finding is connected with the progressive invalidity which leads to muscular atrophy in the extremities. Skeletal dimensions, on the other hand, do not seem to decrease with inactivity (17). Skeletal growth should be normal in the cases studied, as the average age at which the ability to walk is lost is as high as 19.9 years (23). One reason for the reduced blood flow values measured in this study therefore seems to be the changed ratio between soft tissue and bone per unit volume as compared with the normal group. With a view to evaluating the importance of this factor, the flow values for arms and legs at rest and with reactive hyperaemia were recalculated in accordance with the following formula

corrected blood flow value =

$$= \frac{a\,V + b(V_N - V)}{V_N}$$

where

a — measured blood flow value, ml/min./100 ml

Table V Coefficients of correlation for age (1) duration of disease (2) calf blood flow at rest (3) and after arterial occlusion (4) and forearm blood flow at rest (5) and after arterial occlusion (6)

	3 (n = 29)	4 (n = 26)	5 (n = 29)	6 (n = 26)
1	−0 46	−0.44	−0.49*	−0.54
2	−0.57	−0.51	−0.51	−0.55

between each of these two groups and group N ($p < 0.001$) (table IV)

Ganglionic block. The mean values for blood flow at rest and reactive hyperaemia after ganglionic block are given in table III Group N showed an increase in the calf blood flow at rest after block ($p < 0\ 005$) but no other significant changes were noted in the healthy individuals (table IV) The same effect from blocking on calf blood flow at rest was recorded in group I + II ($p < 0\ 001$) and group III + IV ($p < 0\ 002$) The increase in forearm and calf blood flow following arterial stasis was the same with ganglionic block as without, in groups N and I + II. Group III + IV on the other hand showed a significant increase in the reactive hyperaemia in the calf following blocking ($p < 0\ 002$)

Ganglionic block was accompanied by a fall in arterial blood pressure Systolic pressure fell by 11 ± 8 mm Hg ($p < 0\ 001$) and at the same time a tendency was noted towards a higher pulse rate (10 ± 15 beats/min $p < 0\ 05$) Sympathicommetics were administered in four cases after the examination to counter hypotonia and orthostatism.

Correlation between duration of disease age and blood flow data. As apparent from table V the duration of the disease is negatively correlated to both the blood flow at rest and the reactive hyperaemia in calf and forearm. The correlation is similar but less pronounced when age is the independent variable.

2 Pulsations

Oscillography The amplitude of arterial pulsations in calf and forearm for 8 patients in groups III and IV is given in table VI In the Friedreich group the amplitude of forearm pulsations agreed with recordings from 6 normal cases with extremities of comparable circumference. Calf pulsations, on the other hand and the relation between ipsilateral calf and forearm pulsations were significantly reduced ($p < 0.001$)

Toe plethysmography Toe plethysmography was carried out on 6 patients in groups III and IV The time of inclination calculated ad modum Lund (18) was in all cases normal (table VI) as compared with the values measured in 6 healthy children

3 Skin temperature

Table VI shows skin temperature values in patients in groups III and IV after 30 minutes indirect heating and after ganglionic block. The temperature as recorded after indirect heating is significantly lower than that measured in 6 normal cases ($p < 0\ 001$)

4 Atrophy

The individual degree of atrophy was calculated by comparing the segment volumes measured with normal values, the latter being calculated on the basis of age and sex from the tables given by Meredith and Boynton (19) When the volumes measured in the normal group were compared with corresponding calculated normal values, no significant differences could be demonstrated The

disease (d) (table V). Regression analysis with both duration of the disease and end diastolic pressure as independent variables and arm blood flow as the dependent showed, however, that the material is too small to demonstrate significant partial correlations. There is, however, a clear tendency, namely that the correlation between raised filling pressure and arm blood flow ($r_{ab \cdot d} = -0.52$, $r_{ae \cdot d} = -0.60$*) would seem to be real, while the relation between duration of disease and arm blood flow ($r_{ab} = -0.06$, $r_{ab} = +0.06$) is probably only apparent and caused by the correlation between end diastolic ventricular pressure and duration of the disease ($r_{ad} = +0.64$). It is thus likely that patients with Friedreich's ataxia, like other patients with hypokinetic central circulation, have impaired blood flow in the extremities, caused partly by a compensatory increase in sympathetic tone.

Corresponding analyses have been made in respect of the correlation between cardiac index and the blood flow measured in the forearm. Similar significant tendencies could not be found here, probably owing to the changed regional distribution of cardiac output in these patients (23).

Inactivity

Skin circulation is considerably impaired in patients with lesions of the motor neuron and where the extremity in question is wholly or partially immobilized (8). Duff and Shepherd (4) have described reduced blood flow in inactive and chronically denervated forearms. The blood flow there, particularly in the musculature, showed a marked dependence on temperature. The patients in groups III and IV of the present material were often troubled by cold feet and usually a slightly cyanotic skin colour could be observed. It is therefore probable that impaired skin circulation contributed to the decline in blood flow recorded in the legs. These patients, however, enjoy more or less normal activity in the arms. In spite of this, even after correction for atrophy the blood flow values at rest and during reactive hyperaemia are significantly lower than the corresponding normal values.

The marked reduction in calf blood flow in the invalided patients of group III + IV cannot, however, be caused simply by impaired skin blood flow and is propably due mainly to a decreased flow of blood through the musculature. These observations agree with results obtained by Eriksson et al. (5) from arteriography and plethysmographic measurements on immobilized extremities. The muscular wasting was found to be accompanied by a reduced resting blood flow and above all a decreased size of the reactive hyperaemia after arterial occlusion.

Organic arterial changes

A conceivable contributory factor to the circulatory disorder observed, is the presence of obstructive arterial changes in the extremities, in keeping with the submi[...] mal proliferation of coronary arterie[...] described in these patients (13). Th[...] marked circulatory disorder in cases 3[...] and 3 b, both of whom had been sufferin[...] from juvenile diabetes mellitus for som[...] years, can also be suspected of stemmin[...] from diabetic angiopathy. Patient 3 [...] however does not deviate from the oth[...] cases of group III + IV in respect [...] forearm and leg pulsations. The arteri[...] pulsations measured on the forearm in t[...] Friedreich cases are perfectly ordinar[...] accompared with the normal group. T[...]

b = corresponding blood flow recorded in the normal group ml/min./100 ml

V_P = segment volume measured in the pathological group ml

V_N = calculated normal segment volume, ml

This correction compensates the values measured in the Friedreich cases for the atrophied volume. The blood flow in this part is calculated from the values recorded in the normal group Statistical processing of the values thus obtained shows that $p < 0.01$ still applies to all the differences indicated in table IV with $p < 0.01$ or $p < 0.001$ Correction did not influence the differences designated in table IV with $p < 0.05$ It is thus clear that the decline in blood flow cannot be explained solely by the changed relationship between skeletal volume and soft tissue as compared with the normal group Holt (9) making occlusion plethysmographic recordings of *blood flow* in the extremities of patients with hemiplegia and cerebral palsy was unable to demonstrate any correlation between the degree of atrophy and the decline in blood flow

Sympathetic tone

Tachycardia and pronounced orthostatic reaction are frequently found in conjunction with Friedreich's ataxia Studies of the central circulation have shown that the cardiac disease of these patients is characterized above all by an increased filling pressure in the ventricles and a small stroke volume (23) An increased sympathetic tone in these cases could conceivably be a compensatory mechanism designed to maintain an adequate cardiac output. It is on the other hand also possible that a disturbance in sympathetic regulation could be the result of a central nervous lesion.

In conjunction with induced ganglionic block in 5 normal cases an increased blood flow was recorded in the calf at rest but otherwise no significant effects occurred. Patients in groups I + II and III + IV both showed the same increase. Group III + IV however also displayed a significant increase in the extent of the reactive hyperaemia in the leg after ganglionic block. This effect was probably caused by an abnormally high vasoconstrictor tone in the skin — and possibly also in the musculature — before blocking The lower skin temperature after indirect warming as compared with the normal group (table VI) was probably caused by the same phenomenon.

The resting forearm blood flow tended to decrease in all groups following ganglionic blocking (tables III and IV) This could be an effect of the lowered blood pressure and a redistribution of the cardiac output

Donald et al (3) studying patients with rheumatic heart disease, reported an increased axillary a—v O_2 difference at rest as an expression of impaired blood flow in the arm owing to increased vasoconstrictor tone. The fall in the arm blood flow was proportional to the reduction in cardiac output. The skin circulation is affected first, the muscle blood flow decreasing only in severe heart disease (20) On the basis of data obtained from the heart catheterization of 15 patients in the present material there has been found significant correlations between the end diastolic pressure in the right ventricle (a) and the size of both blood flow at rest (b) ($r_{ab} = 0.63^{**}$ n = 16) and reactive hyperaemia (c) ($r_{ac} = -0.68^{**}$ n = 15) in the arm. All these factors, however are also correlated to the duration of the

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calf pulsations, on the other hand and with these the quotient of the calf and arm pulsations, are significantly reduced. Factors contributing to the deviation from the normal group would seem to be (a) that both the relative and the absolute volume of muscle tended to be greater in the leg segment in the normal group and (b) that there is an increased sympathetic tone in the legs of the Friedreich group. Small pulsations were also recorded at digital plethysmography but the calculated time of inclination is perfectly ordinary. It is therefore less likely that the circulatory disorder is caused by stenotic arterial changes.

Summary

1. In 29 cases of Friedreich's ataxia, grouped according to degree of physical handicap (groups I—IV) and in 10 healthy subjects blood flow in forearm and calf was measured at rest and during reactive hyperaemia. In 13 Friedreich cases and 5 healthy subjects blood flow was also registered after ganglionic blocking. Oscillography, digital plethysmography and skin temperature measurements were performed in 8 cases from groups III and IV and in 6 healthy subjects.

2. In comparison with the findings in the healthy subjects blood flow was reduced in both forearm and calf at rest and during reactive hyperaemia in the groups where ability to walk was lost (III and IV). Similar findings were made in groups I and II but the reduction in blood flow was less pronounced. Significant reductions of blood flow remained after correction for estimated degree of atrophy.

3. Following ganglionic blocking a significant increase in the size of the reactive hyperaemia was noted in groups III and IV.

4. Definite signs of obstructive arterial disease could not be demonstrated.

5. The cause of the decreased blood flow in the extremities in Friedreich's ataxia is thought to be due to muscular atrophy and inactivity and to an increased vasoconstrictor tone in skin and muscle. A relationship between the sympathetic tone and degree of myocardial dysfunction is suggested.

Acknowledgement

The present investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Cold Agglutinins in Pneumonia

By

Elli Jansson and Odd Wager

Cold agglutinins ha e been observed in a ariety of diseases, e.g trypanosomiasis, liver diseases and blood dyscrasias. The first to establish them in a patient with pneumonia were Clough and Richter in 1918. Peterson et al. in 1943 observed a high incidence of cold agglutinins in primary atypical pneumonia (9) The figures reported in later studies ha e rang ed from 30 to 90 per cent (11)

Many investigators have established a correlation between the occurrence of a high cold agglutinin titre in patients with pneumonia and the fever duration and severity of the disease (9 10 12) A case of pneumonia associated with high cold agglutinin titre (1 1024) and resulting in hemoly tic anemia with a fatal outcome has also been described in the literature (4)

A number of authors have bserved cold aggl tinins in pneumonias caused by various etiological agents, such as influenza irus (2) psittacosis virus (9) adenovirus (8) Eaton gent (7) R. burnetii and Diplococcus pneumoniae (1) Some investigators are of the opinion that most ases of cold agglutinin-positive pneumo ia are caused by Eaton PPLO Mufson et al. studying a military population, found a fourfold rise in cold agglutinins in 45 per cent of Eaton agent pneumonia in contrast to 17 per cent of cases of adenovirus pneumonia and 6 per cent of atypi cal pneumonias of unknown etiology (8)

This paper is a report on the results of our studies of cold agglutinins in patients with pneumonia who were treated at Aurora Hospital, Helsinki.

Material and methods

The series consisted of 246 patients with pneumonia hospitalized between mid-September 1962 and end-April 1963. A more detailed description of this patient series is given elsewhere (5)

Blood samples were taken from the patients on admission and then t weekly intervals throughout the hospital stay From the cases classified by serological criteria as Eaton PPLO pneumonia an additional blood sample was requested in April-May 1963. The control series comprised the sera of 484 blood donors collected during April—May 1963.

T prevent the disappearanc of cold ag glutinins from the sera, the blood samples were not placed in a refrigerator before they were sent to the laboratory After separation, the serum samples were stored at + 4 C until

Submitted for publication November 27 1963.

Table I. Age distribution of patients with pneumonia and of blood donors

Age (yrs)	Patients with pneumonia			Blood donors		
	Total	Cold agglutinins ≥ 32		Total	Cold agglutinins ≥ 32	
		No.	%		No.	%
<3	20	4	20	—	—	—
3—5	36	13	36	—	—	—
6—10	42	27	64	—	—	—
11—15	29	24	83	—	—	—
16—20	17	12	71	9	—	—
21—30	15	4	27	146	5	3.4
31—40	11	3	27	116	1	0.9
41—50	24	7	29	103	5	4.9
51—60	21	2	9	83	5	6.0
61—70	14	3	21	26	1	3.8
>70	17	3	18	1	—	—
	246	102	42	484	17	3.5

Table II. Patients with pneumonia distributed by month of onset

	Total	Cold agglutinins ≥ 32	
		No.	%
1962			
Sept.	13	6	46
Oct.	31	15	48
Nov.	57	21	37
Dec.	39	17	44
1963			
Jan.	31	13	42
Feb.	27	10	37
March	25	12	48
April	23	8	35

Table III. Patients with pneumonia and blood donors distributed by the cold agglutinin titre

Cold agglutinin titre	Patients with pneumonia		Blood donors	
	No.	%	No.	%
<4	78	32	294	61
4	22	9	102	21
8	29	11	55	11
16	15	6	16	3
32	17	7	13	3
64	24	10	3	1
128	15	6	1	—
256	35	14	—	—
512	10	4	—	—
1,024	—	—	—	—
2,048	1	—	—	—

the third week in 6 patients. The maximum titre was reached rapidly in 19 patients in the second, in 20 in the third week, and in 2 later. Disappearance was established in 8 cases, in the third week in 2, in the fourth week in 3 cases, in the fifth in 1 case and in the sixth in 2 cases.

In the follow-up examination of 13 patients with cold agglutinin-positive pneumonia 3—5 months after onset of illness the titres for cold agglutinins were < 4 in 4, 4 in 3, 8 in 4 and 64 in 2 cases, and in 11 patients studied 6—7½ months after onset: 4 in 5 cases, 8 in 1 case, 16 in 4 cases and 32 in 1 case.

Table III gives the distribution of the series by the highest cold agglutinin titre. The titres were generally high in cold agglutinin-positive pneumonia, ≥ 128 in 60 and ≥ 512 in 10 per cent of the cases. Only 4 blood donors had a cold agglutinin titre of ≥ 64. Such high titres were thus established almost exclusively in patients with pneumonia.

Relation to Eaton pneumonia. The incidence of Eaton pneumonia in 102 cases of cold agglutinin-positive pneumonia was 26 per cent and in 144 cases of pneumonia without cold agglutinins 9 per cent. As can be seen from fig. 3, the peak incidence of cold agglutinin-positive pneumonias as well as of Eaton pneumonias occurred in the age group 6—15 years.

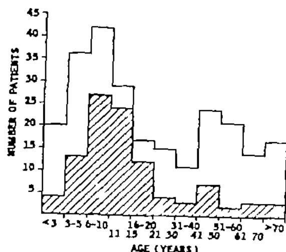


Fig 1 Age distribution of patients with pneumonia.
☐ All patients with pneumonia
▨ Patients with cold agglutinin titre ≧ 32

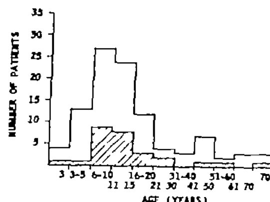


Fig 3 Patients with cold agglutinin-positive pneumonia distributed by age and Eaton PPLO etiology
☐ All patients with cold agglutinin-positive pneumonia
▨ Patients with Eaton pneumonia

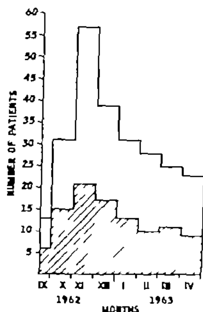


Fig 2 Patients with pneumonia distributed by month of onset.
☐ All patients with pneumonia
▨ Patients with cold agglutinin titre ≧ 32

tested. The cold agglutinins were studied according to Feller and Hilleman (3) Human O cells older than 5 days were not used. Examination for cold agglutinins was carried out 2–4 times a week.

Eaton PPLO antibodies were studied by the CF test, employing the technique previously described (5)

As "cold agglutinin positive" were regarded the cases with a cold agglutinin titre of ≧ 32. As "Eaton pneumonia" were classified the cases which fulfilled the serological criteria reported in detail elsewhere (5)

Results

Of a total of 246 patients with pneumonia, 102 or 42 per cent had cold agglutinins in a titre of ≧ 32. The corresponding figure for 484 blood donors was 17 or 3.5 per cent. The difference is highly significant.

Age distribution The incidence of cold agglutinin positive pneumonia was highest in the age groups 6—20 (fig 1 and table I) In patients under 16 years of age the figure was 54 per cent, and in those over 16 years 29 per cent. This difference is highly significant

Seasonal distribution It can be seen from fig 2 and table II that cold agglutinin positive pneumonias were equally distributed over the 8 months studied.

Pattern of cold agglutinins In 18 cases with no cold agglutinins (titre < 4) in the first week of hospitalisation agglutinins appeared in the second week in 12 and in

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16	15	6	16	3
32	17	7	13	3
64	24	10	3	1
128	15	6	1	—
256	35	14	—	—
512	10	4	—	—
1 024	—	—	—	—
2,048	1	—	—	—

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Comments

The 42 per cent incidence of cold agglutinin positive pneumonia established in the present series agrees with earlier observations. Savonen in his extensive study in Helsinki in 1946 found a cold agglutinin titre of ≧ 32 in 36 per cent of adult patients with pneumonia (10) as compared with 29 in our investigation. The corresponding figures for the control groups were 4 5 per cent and 3 5 per cent respectively

The predominance of cold agglutinin positive cases in the young age groups in the present series is in accordance with the results obtained by Knutsen for a material of similar type (6) The peak incidence of cold agglutinin positive pneumonia and that of Eaton pneumonia fell in the same age group 6—15 years, in our series

The even distribution of cold agglutinin positive pneumonia over 8 months established here accords with the view that cold agglutinin-positive pneumonia is not etiologically uniform. Possibly the appearance of cold agglutinins in patients with pneumonia is due rather to the extent and type of the lesion than to a certain etiologic agent (10) It would in fact be interesting to know more about the possible occurrence of cold agglutinins in other human diseases.

Summary

Of a total of 246 patients with pneumonia, 42 per cent had cold agglutinins in a titre ≧ 32 The corresponding figure for 484 blood donors was 3 5 per cent Titres ≧ 64 were seen practically only in patients with pneumonia.

The incidence of cold agglutinin positive pneumonia was 54 per cent in patients under 16 years and 29 per cent in those over 16 years. No seasonal variation could be observed.

The peak incidence of cold agglutinin positive pneumonia and Eaton PPLO pneumonia fell in the same age group 6—15 years. The incidence of Eaton pneumonia in cases with cold agglutinin positive pneumonia was 26 per cent and in those without cold agglutinins 9 per cent.

Acknowledgement

Aided by a grant from the Sigrid Jusélius Foundation. The technical assistance of Miss Sirkka-Liisa Tuuri is gratefully acknowledged.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Hypercalcaemia Treated with Sodium Phytate

By

E. Roelsen and Leif Paulsen

In 1956, Henneman et al. (5) reported that after administration of sodium phytate the serum calcium returned to normal in three patients who presented hypercalcaemia in association with sarcoidosis. This effect of the drug was ascribed to reduced intestinal absorption of calcium, and, possibly, to a suppression of the parathyroid function through increased phosphate absorption. The authors concluded that sodium phytate is "the treatment of choice" of hypercalcaemia in sarcoidosis and suggested its use in vitamin-D intoxication, idiopathic hypercalcuria and infantile hypercalcaemia. Such conditions were previously and are presumably still, treated with corticosteroids.

Considering that phytic acid has been known for a long time as a rachitogenic anti-vitamin D occurring in various cereals, it is surprising that sodium phytate only relatively recently has been used in the treatment of hypercalcaemia referable to vitamin-D effect. Mollgaard (6) has extensively studied the significance of this substance in cattle breeding and manufacture of rye bread in Denmark.

Submitted for publication November 28, 1963.

Phytic acid is inositol hexaphosphoric acid, which according to Henneman et al. has the theoretical formula shown in fig. 1.

Oral administration of the sodium salt of phytic acid results in increased faecal excretion of calcium, presumably because of the formation of unabsorbable complex calcium phytate compounds, while the urinary excretion of calcium is simultaneously decreased. However sodium phytate or its complex compounds are partially hydrolysed, resulting in a simultaneous increase in the phosphate absorption.

Three cases of hypercalcaemia treated with sodium phytate are reported below. This was given in the form of Rencal® (Squibb Sodium Phytate) which is dispensed as cola-flavoured granules in doses of 4.5 g providing 3.0 g sodium phytate or as a powder of which 10 ml provide 3.0 g sodium phytate.

Case reports

Case 1. A married woman aged 49 was admitted to the Central Hospital, Silkeborg for the first time on Aug. 17, 1961, and several

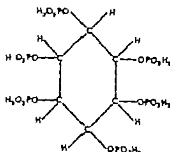


Fig 1 Theoretical formula of phytic acid, molecular weight 660 (after Henneman et al.)

times afterwards. She had previously experienced periods of depression. The menopause had occurred at the age of 47 In the autumn of 1960, the patient had consulted a dermatologist because of some small nodules in the skin of the forehead. Biopsy revealed sarcoidosis. She was then treated with concentrated calciferol, 6 drops daily i. e. about 60,000 I U vitamin D_2, for 6–7 months. In the spring of 1961 symptoms of disc prolapse developed, for which reason she took fairly large amounts of codesphen® for some months. She was then admitted to the County Hospital, Hammel, with symptoms of fatigue and depression and sciatic pain. The cervical, axillary and inguinal lymph nodes were enlarged. Chest radiography revealed a swelling of the upper part of the mediastinum (measuring 8 cm in width) presumably on account of lymph-node enlargement. A biopsy specimen from an inguinal lymph node showed sarcoidosis. Mild normochromic anaemia (Hb 80–60 %) was present E. S R. 60–75 mm/hour tuberculin reaction negative The temperature was only slightly elevated. Serum calcium 13.9–13.8 mg/100 ml serum phosphate 5.8 mg/100 ml (Normal values in our laboratory for serum calcium 8 6–10 6 mg/100 ml (flame photometry Eppendorfer) and for serum phosphate 2 7–4 7 mg/100 ml (colorimetry Fiske-Subbarow)) Apart from lumbar disc degeneration, radiography failed to reveal osseous changes. The urine was negative for protein and sugar with normal microscopy Urinary output about 2 litres specific gravity 1 003–1 010 Serum creatinine 3 mg/100 ml. Blood pressure 160/100 mm Hg ECG showed left axis deviation and a few ventricular extrasystoles.

On admission to the Central Hospital, she was still tired and depressed, but the sciatic pain had abated after prolonged extension therapy The enlargement of the peripheral and mediastinal lymph nodes was unchanged. Serum calcium 14 6 mg/100 ml serum phosphate 5 1 mg/100 ml alkaline phosphatase 7.8 King Armstrong units/100 ml thymol 8.1 units/100 ml serum creatinine 2.8 mg/100 ml creatinine clearance in 24 hours 34 ml/min. Blood pressure 190/110–150/100 mm Hg Serum electrophoresis showed increased gammaglobulin (2.2 g/100 ml) Radiography did not show any signs of nephrocalcinosis.

Diagnosis Nephropathy referable to sarcoidosis with hypercalcaemia (?) or to excessive medication of calciferol (?) and interstitial nephritis caused by phenacetin (???)

Treatment and course

The patient was up and about during the greater part of the hospital stay The dosage of sodium phytate appears from the diagrams in figs. 2 and 3 For periods, she was placed on a standardised diet, low in calcium and phosphate (Snapper) otherwise she was on a slightly modified hospital diet.

The Snapper diet is very suitable as a standard for the assessment of the calcium and phosphate metabolism. Its composition is shown in table I

Fig 2 shows a high initial serum calcium and a slightly increased phosphate level. The urinary excretion of calcium was slightly elevated or at the upper limit of normal (200–235 mg) and the phosphate excretion was about 770 mg/24 hours. During this period the patient was on an unrestricted diet. On Snapper diet, the calcium excretion was depressed to about 150 mg/24 hours, and administration of sodium phytate resulted in a prompt fall to about 50 mg/24 hours. At the same time th phosphate excretion increased distinctly to 1 700 mg/24 hours. The serum calcium returned to normal, from 13.4 to 8.8 mg/100 ml while the serum phosphate showed an appreciable rise from 4.8 to 8.0 mg/100 ml.

During about 4 months continuous treatment with sodium phytate, 3 g three times daily while the patient was on an unrestricted diet (yet without milk, cheese and cabbage th serum calcium remained essentially normal The patient then wanted to continue on the same diet, but with only 3 g sodium phytate twice daily From March 26, 1962,

Table I. Snapper diet containing 202 mg calcium and 272 mg phosphate

	Calories
6 rusks	150
100 g rice	300
75 g butter	600
3 eggs	300
200 g beef tea	50
2 cups of tea (400 g) with sugar	50
2 oranges	100
200 g fruit juice	50
Total	1,600

we managed to get an unbroken experimental period of 42 days. During this period, the patient, who co-operated satisfactorily, was mainly observed in our out-patient clinic. The pertinent clinical data are shown in fig. 3.

At first, after less intensive treatment for little less than 4 months, an increase in serum calcium to 11.4 mg/100 ml and a decrease in serum phosphate to 4.8 mg/100 ml occurred, accompanied by normal or slightly increased urinary calcium excretion of about 250 mg/24 hours, while urinary phosphate remained at approx. 1,500 mg/24 hours. It also appears from the diagram that the Snapper diet as such gave distinct decrease in urinary calcium. The dose of sodium phytate and the calcium excretion showed good correlation. Between the 29th and 34th days inclusive sodium phytate was tentatively withdrawn. This resulted in prompt increase in the calcium excretion from 150 to 450 mg/24 hours, and at the same time the serum calcium increased to 1[illegible] mg/100 ml. During the next period the dose of sodium phytate was increased to 5 g four times daily; the calcium excretion then showed steep fall to about 75 mg/24 hours, and the serum calcium returned to normal, 10 mg/100 ml.

The phosphate excretion was almost inversely related to the calcium excretion, as shown in the first diagram (fig. 2).

The percentage of tubular reabsorption of phosphate (TRP[illegible]) was calculated in several experiments both during and without administration of sodium phytate. The results appear from table II.

Subjectively, the patient felt much better when the serum calcium returned to normal.

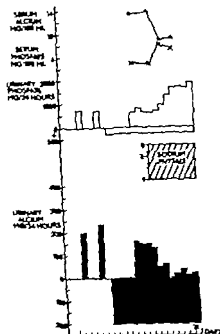


Fig. 2. Case 1. Preliminary observation period. Both in this and the following diagrams the amounts of phosphate and calcium in the Snapper diet are indicated by white and black rectangular area, respectively below the base line (0). The figures along the hatched sodium phytate area indicate the daily dose in g.

At first, the ingestion of the drug gave rise to mild cardialgia and a burning sensation in the mouth (considerable heat of hydration) accompanied by meteoristic rumbling and fairly loose stools. However, when the patient took the drug dissolved in a small glass of ice water during a meal, these side effects became negligible. Only during the period with dose of 5 g four times daily did signs of enteritis develop. Anaemia (iron was given all the time) or electrolyte disturbances did not occur. The serum magnesium concentration remained constant, at a level abov[illegible] 1.7 mEq/l. After treatment for 16 months, radiography did not show any signs of halisteresis. When, in January 1963, the therapy was tentatively discontinued, serum calcium rose to 11 mg/100 ml, and the urinary excretion increased at once, while urinary phosphate fell. At the same time, the patient felt tired and

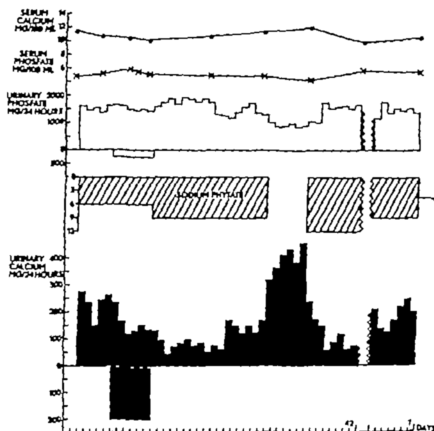


Fig 3 Case 1 Long term observation period. The interval between the two experimental periods (42 and 7 days) was 8 days.

depressed. As sodium phytate has not yet been approved as subsidised drug in Denmark, it was suggested that the patient should be admitted for a course of corticosteroid therapy

Repeated examinations during the spring and summer of 1963 showed normal serum calcium values, whereas the urinary calcium excretion had again risen to, or occasionally even exceeded, the level observed at the institution of treatment in August 1961 During a hospital stay in March 1963 the serum creatinine was 1.9 mg/100 ml and the creatinine clearance in 24 hours was 43 ml/min The TRP had risen to 70 E. S. R. 27 mm/hour Hb 97 % Serum electrophoresis showed normal conditions. Blood pressure 125/95 mm Hg

The general condition was greatly improved The enlarged peripheral lymph nodes had decreased in size, whereas the mediastinal swelling remained unchanged. The aforementioned nodules in the skin of the forehead had begun to recur The contemplated corticosteroid therapy was not instituted.

Case 2 A farmer aged 48, was admitted to the Central Hospital, Silkeborg, for the first time on Oct. 28, 1961 and several times afterwards. Since the age of 12, the patient had suffered from recurrent urolithiasis. At first, there were symptom-free intervals of several years, while, during recent years, these intervals had often extended over only from 2 to 6 months. About 20 years ago, ureterolithotomy was performed in the County Hospital, Hammel. In 1961 he was admitted to the same hospital with bilateral ureteral colic and haematuria. Urography revealed a stone, 0.5 × 2 cm, in the left renal region and another 2.5 cm in diameter in the right renal region. Bilateral hydronephrosis was present A varying degree of acidosis was observed Isosthenuria was present serum creatinine 1 6 – 1 3 mg/100 ml. The urine contained protein, but no sugar microscopy showed pyuria of variable severity Serum calcium 12.8 mg/100 ml serum phosphate 2 8 mg 100 ml alkaline phosphatase 9 4 – 5.8 – 11 0 King-Armstrong units/100 ml. The

Table II. The TRP% in six experiments in case 1

Date	TRP%	Creatinine clearance in 24 hrs (ml/min)	Type of diet	Daily dose of sodium phytate (g)
1962				
6/4	51	38	Snapper	6
1/5	53	31	Modified diet	6
9/5	34	30	Modified diet	12
21/5	46	34	Modified diet	9
23/10	47	36	Modified diet	9
28/10	67	34	Modified diet	0

See text.

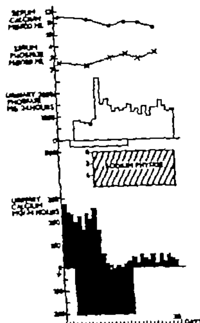


Fig. 4. Case 2. Preliminary observation period. Note that the scale for the serum levels of calcium and phosphate is twice as large as in figs. 2 and 3.

stones removed by pyelolithotomy and nephrolithotomy consisted of calcium oxalate and calcium magnesium phosphate. The blood pressure was normal. ECG showed left axis deviation. Radiography did not show any signs of osseous changes.

Diagnosis. Idiopathic infantile hypercalcaemia.

Treatment and course

The serum levels of calcium and phosphate responded qualitatively but not quantitatively as in case 1 while the urinary excretion of the two minerals was of the same order of magnitude (fig. 4). In this experiment, no distinct effect of the Snapper diet was observed.

During prolonged observation period from Jan. 19 1962, the urinary excretion of calcium was constantly lower, especially on Snapper diet, than in case 1 (fig. 5). On withdrawal of sodium phytate for 9 days an increase in urinary calcium occurred, although it was smaller than in case 1. The phosphate excretion was slightly lower than in case 1. The serum calcium did not convincingly return to constantly normal level, and the serum phosphate was normal. Oral administration of a single dose of vitamin D_2 (50,000 I. U.) was followed by increases in the serum levels of both calcium and phosphate. These observations will be discussed later. After treatment for 14 months, radiography did not reveal signs of osseous changes.

The patient was often troubled by cystopyelitis accompanied by dysuria and pollakisuria, which required intermittent or prolonged treatment with antibiotics or sulphonamides. During the period of treatment with sodium phytate, large urinary calculi did not develop, but small concretions were passed on a few occasions. Now and then the patient suffered from enteritis with hypopotassaemia and falls in the serum magnesium to 1.1 mEq/l, without concurrent characteristic neurological manifestations.

The determinations of the TRP% in this patient appear from table III.

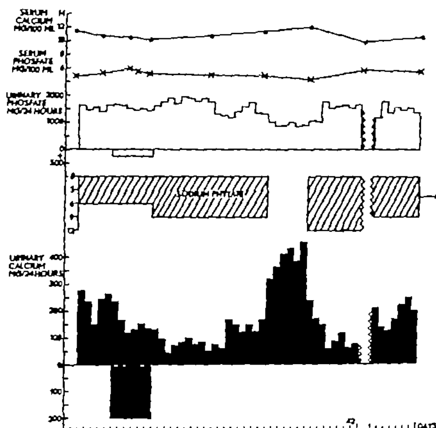


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in the serum phosphate level and a slightly increased primary excretion of phosphate. Accordingly definite diagnosis of hyperparathyroidism was made.

One month later besides a normal parathyroid gland an adenoma measuring 0.5 × × 2 cm, posterior to the right lobe of the thyroid gland, was removed in the County Hospital, Hammel. The adenoma was histologically verified. Daily determinations of the serum levels of calcium and phosphate during the last six days before the operation showed average values of 11.1 and 3.0 mg/100 ml, respectively. The corresponding average figures for the first six postoperative days were 8.8 and 3.7 mg/100 ml. During the same periods, the 24-hour excretion of calcium and phosphate averaged 221 and 398 mg before and 93 and 497 mg after operation.

Case 3. A married woman aged 24, was admitted to the *Central Hospital, Silkeborg*, on July 20, 1963. In 1955, a toxic goitre had been diagnosed, and in 1956, subtotal thyroidectomy was performed in another hospital, according to the patient's statement because of pressure symptoms. After the operation, partial paralysis of the recurrent nerve occurred, and tetany developed in 1960. She was then treated with concentrated calciferol. from June 1961 she was given 10 drops twice daily. She had given birth to two children, in 1960 and in mid-July 1963. During the last five months of the last pregnancy the calciferol dose had been increased by the family doctor to 15 drops twice daily (i.e. approx. 300,000 I.U. vitamin D_2) but the serum calcium level had not been checked. The patient was clinically well until the second day post partum, when syncope, nausea, vomiting, anorexia, polydipsia and polyuria occurred. The baby was normal.

Physical examination on admission revealed a rather weak patient. Serum calcium 13.8 mg/100 ml, serum bicarbonate 29.3 mEq/l, serum potassium 3.2 – 3.0 mEq/l. Blood pressure 195/120 – 160/100 mm Hg. The urine was first positive, later negative for protein. Serum creatinine 1.5 – 1.8 mg/100 ml. Ophthalmoscopy revealed retinopathy of pregnancy. Treatment with sodium phytate powder 15 ml three times daily (i.e. 13.5 g) was instituted at once. In addition, potassium chloride and saline were administered. Within two days, the serum calcium level returned to normal (10.2 mg/100 ml) and just as in the first two cases, the urinary excretion of calcium decreased. Tentative withdrawal of the drug four days later resulted in an increase in serum calcium to 11.6 mg/100 ml. After treatment for another 12 days, sodium phytate could be discontinued. One month later the patient was discharged with normal serum levels of creatinine and calcium for continued observation and treatment in our out patient clinic.

Discussion

Treatment of hypercalcaemia with sodium phytate results in a reduction in the intestinal calcium absorption. The diagrams seem to be convincing. In all three cases a considerable decrease in the urinary excretion of calcium was obtained especially in cases 2 and 3. The serum calcium level returned to normal in cases 1 and 3 and it was also depressed in case 2, although the depression was not conspicuous in the prolonged course of treatment. For technical reasons, it was not possible to determine the faecal excretion of calcium. However Henneman et al. (5) observed a distinct increase in faecal calcium during administration of sodium phytate to two patients with sarcoidosis associated with hypercalcaemia, but strangely enough, this phenomenon could not be demonstrated in the third of their patients.

In case 1 hyperparathyroidism may presumably be ruled out, even though the combination of that condition and sarcoidosis has been described as an extremely rare occurrence (2). In this patient the hypercalcaemia may be explained as being due to generalised sarcoidosis, which had been confirmed by biopsy specimens taken from the skin and a lymph node. It may be assumed that the granulomatous tissue forms vitamin-D-like substances. In the literature, some

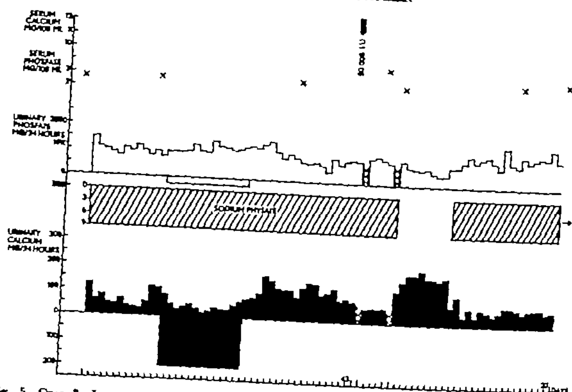


Fig 5. Case 2 Long-term observation period See also text in fig 4 The interval between the first and second experimental periods (43 and 4 days) was one day and that between the second and third periods (4 and 27 days) was 48 days.

Table III The TRP% in four experiments in case 2

Date	TRP%	Creatinine clearance in 24 hrs (ml/min)	Type of diet	Daily dose of sodium phytate (g)
1962				
2/5	72	47	Modified diet	0
15/5	61	51	Modified diet	9
1963				
20/1	88	42	Modified diet	0
24/1	64	50	Snapper	9

See text.

At a reappraisal of the condition after the discontinuance of sodium phytate therapy during a hospital stay in May 1963 hypercalcaemia was still present. On 12 consecutive days during which the patient was on an unrestricted diet (yet without milk, cheese and cabbage) for the first nine and on Snapper diet for the last three days, the following values were obtained. Serum calcium 11.8 – 11 6 – 11 4 – 11.6 – 11 0 – 10.8 – 10.8 – 11 4 – 12 4 – 10 4 – 11 6 and 10.6 mg/100 ml. Serum phosphate 2.5 – 2.3 – 2.7 – 2.2 – 2 9 – 3 1 – 3.2 – 3 8 – 2.9 – 2 9 – 2.5 and 2 9 mg/100 ml. Apart from two values of 2.5 and ? 6 mg/100 ml, the serum phosphate level had not previously been definitely decreased i e below 2 7 mg/100 ml. As no less than four of the values just stated were below that lower limit of normal, this observation and other considerations (see below under Discussion) were found to justify a calcium tolerance test, in which 15 mg calcium per kg body weight was given intravenously within 6 hours. This test revealed absence of a normal increase (i. e. above 1 mg)

in the serum phosphate level and slightly increased urinary excretion of phosphate. Accordingly definite diagnosis of hyperparathyroidism was made.

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diversity of opinion has been expressed as to the frequency of hypercalcaemia in sarcoidosis it has been variably reported to be from a few up to 20—30 % (1 6) Hypercalcaemia associated with sarcoidosis leading to the formation of renal calculi must be presumed to be rare. Among about 100 patients with sarcoidosis, we have only seen one such case. James (7) expressed the opinion that hypercalcaemia in sarcoidosis is induced by calciferol in most cases, but this view is challenged by other authors (1) In this connexion it may be mentioned that the three patients described by Henneman et al. prior to the disclosure of hypercalcaemia had been treated with vitamin D although in low dosage.

That our patient had been treated with vitamin D was due to the fact that this therapy which was introduced in the 1940 s in analogy with Charpy s vitamin D therapy of lupus vulgaris (4) is — or at least has been — widely used The results seem to be good but it was expressly pointed out by Gilg that these patients are more susceptible and more liable to vitamin D intoxication than patients with lupus vulgaris Just because of its potential hypercalcaemia producing effect, possibly in a special group of patients this therapy should presumably be instituted and maintained only under very strict control. It cannot be excluded that the hypercalcaemia in our patient was induced by the large total dose of calciferol but if so its effect must have been of an exceedingly protracted character We shall revert to this problem later

It is usually assumed that hypercalcaemia due to vitamin D may be induced by two different modes of action 1) increased intestinal absorption of calcium, and 2) decreased tubular reabsorption of phosphate. As previously pointed out, administration of sodium phytate counteracts 1) whereas the increased phosphate concentration in the serum counteracts 2) by a homeostatic mechanism through the parathyroid glands. The former effect exerted by sodium phytate is presumably the most important.

Repeated examinations in case 1 showed that the TRP % was reduced, most pronounced during periods with administration of sodium phytate. This reduction need not necessarily be a manifestation of hyperparathyroidism, but may be explained by a tubular lesion constituting part of the renal disease in the patient. The renal function was reduced to one third of normal and remained surprisingly constant for more than 12 months until the termination of treatment. Three months later some improvement was observed The impaired renal function was presumably due to hypercalcaemia as such even though radiography failed to reveal signs of nephrocalcinosis. However it may also be conceived that the sarcoid processes as such may have been of importance in this development. It cannot be excluded that the intake of large doses of phenacetin has had a certain effect, although this seems to be less likely The anaemia was not severe, and there were no dysuric reactions, as are usually seen in patients with tablet disease of the kidneys

As appears from the diagram in fig 3 eight months after the institution of sodium phytate treatment, i. e. about 12 months after the discontinuance of vitamin D therapy the drug was tentatively withdrawn for six days. This resulted in a prompt increase in urinary calcium and the serum calcium simultaneously rose to a higher level. This phenomenon suggested that the pathological process

was perhaps still active, so that an endogenic vitamin D-like substance was given off from the sarcoid granulomatous tissue. However it is nevertheless conceivable that this effect could be ascribed to the preceding vitamin-D therapy. This view is supported by the fact that after the lapse of two years the patient did not present hypercalcaemia in spite of the withdrawal of sodium phytate, while, on the other hand, she still had clinical symptoms of sarcoidosis (lymph-node enlargement and sarcoid of the skin) although a considerable improvement had occurred. The patient belonged to the group in which sarcoidosis involves other organs in addition to the lungs, and presumably suffered from generalised sarcoidosis. The prognosis seems to be less favourable in this group of patients (4, 9). It is likely that her renal disease would have progressed if she had not received sodium phytate for a prolonged period. Corticosteroid therapy over 16 months would have involved hazards and disadvantages and probably would have been less effective (cf. Henneman et al. (5)).

Originally we interpreted the hypercalcaemia in this patient as a manifestation of a lifelong process which would require continuous treatment with sodium phytate or corticosteroids. Judging from the course of the disease, it seems most likely that the condition was a protracted vitamin-D intoxication which persisted for two years. Correlated with the experience reported by Henneman et al. the course in our patient lends support to James's conception of the relation between hypercalcaemia and sarcoidosis. In another patient with sarcoidosis who had taken small doses of vitamin D preceding admission to hospital, we also observed protracted hypercalcaemia for two years. In that case, sodium phytate could not be given because the drug was not available at that time, but good effect was obtained by means of small doses of corticosteroids. On the whole, these patients (possibly forming a special group) seem to be very sensitive to vitamin D, which by an unknown mechanism may possibly start a further production of vitamin D-like substances (in the granulomatous tissue (?)).

A detail which seems to be of great theoretical interest, but which has not been studied is the phenomenon that, before the institution of treatment, the urinary excretion of calcium was normal or only slightly increased, whereas the hypercalcaemia was considerable, and that, after the termination of treatment, the urinary excretion of calcium was still on the same level, while the serum calcium remained normal. In this connexion we disregard the aforementioned greatly increased urinary excretion of calcium which was observed after the temporary withdrawal of sodium phytate during the long-term experiment, because this increase may at least partially be due to a "rebound" phenomenon. It is beyond doubt that the hypercalcaemia was originally started by a vitamin-D effect. As already mentioned, this effect is traditionally ascribed to an increased intestinal absorption of calcium, but it may also be referable to increased resorption of calcium from the osseous system. In view of the relatively short observation period, the absence of radiographically demonstrable halisteresis does not say anything in this connexion. But it is rather surprising that the patient, both with a markedly increased calcium level and with a normal content of calcium in the serum, presented nearly the same urinary excretion. According to Henneman et al., the urinary excretion of calcium in sarcoidosis associated with hypercalcaemia is markedly increased. That this phenomenon was not very distinct in our case may be due to certain factors which have somehow inhibited or reduced the citrate formation in the plasma at the time when the hypercalcaemia was very pronounced (as to the importance of citrate formation in urinary excretion of calcium, see Fourman (3)). The problem may perhaps be explained simply

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by the impaired renal function which remained essentially unchanged during the entire experimental period, but improved somewhat after the termination of treatment. As previously pointed out, the conspicuous effect of the treatment with sodium phytate obtained in this case was due to the blockage of one of the routes along which the blood content of calcium is renewed.

In case 2 the hypercalcaemia was difficult to classify Stone formation had occurred ever since the childhood of the patient The serum levels of phosphate and alkaline phosphatase were normal The renal function was reduced to about 50 % possibly due to the hypercalcaemia itself but presumably also because of recurrent urinary tract infection Primarily the patient might well be presumed to suffer from hyperparathyroidism, although this on further consideration seemed less likely In spite of the long history of disease, there were no osseous changes, as should then have been expected. The TRP % was apparently normal during periods when no sodium phytate was administered This observation also weighed against a diagnosis of hyperparathyroidism. In this patient the hypercalcaemia was primarily classified as the so-called idiopathic infantile variety which is usually ascribed to an "endogenic vitamin D-like effect or to hypersensitivity to vitamin D We were aware that this disease is rare, but at that time we did not know that cases of it with such a protracted course have presumably never been described In this case, the excretion of calcium was apparently normal or only slightly elevated but yet large enough to give a tendency to stone formation.

On experimental administration of a single dose of 50,000 I U of vitamin D_2, the serum calcium increased promptly from 10 7 to 12 2 mg/100 ml This reaction was suggestive of hypersensitivity to vitamin D and to some extent, in favour of a diagnosis of idiopathic hypercalcaemia. The increase in serum calcium observed in this experiment must presumably be ascribed to resorption of calcium from the bones. The concurrent small increase in serum phosphate may be explained by a so-called masking of the second mode of action of vitamin D i e. a suppressive effect on the parathyroid function Strangely enough, the urinary excretion of calcium did not increase in this experiment. A possible defect in the citrate formation in the plasma may explain this phenomenon.

It must be added that the lower but yet normal serum phosphate level observed in case 2, as compared with the slightly increased values in case 1 cannot be explained by a difference in the TRP % which was higher in case 2 The glomerular filtration was also higher in case 2 which may give a partial explanation of this phenomenon. The discrepancy between the two patients, correlated with the definitely decreased serum phosphate values which were gradually detected and especially the less conspicuous effect of sodium phytate on the serum calcium, led to a revision of the diagnosis. That the phosphate excretion in the long-term experiment in case 2 was less than in case 1 may be explained by the presence of a smaller amount of phytase in the intestinal tract

In case 2 the urinary excretion of calcium also increased after the withdrawal of sodium phytate, although the increase strangely enough, was less than in case 1 The concurrent hypercalcaemia may possibly have been caused by a prolonged effect of the aforementioned single dose of vitamin D_2, which had however been given 48 days previously

It must be admitted that the discussion of these studies could have been conducted with far greater precision if the observation and treatment of the patients had been accompanied by proper balance analyses, i. e. including determination of the faecal excretion of calcium and phosphate. Such determinations would have given a better impression of the relation between the shares contributed by intestinal absorption and bone resorption to the amounts of calcium and phosphate measured in blood and urine.

Case 2, which was unfortunately primarily misdiagnosed and therefore does not belong to the group of patients in whom sodium phytate therapy is appropriate, presents, however several features of didactic and theoretical interest. These may be summarized as follows a) The case is an additional example of the now generally accepted concept that hyperparathyroidism may exist without characteristic osseous changes, provided that the daily intake of calcium is sufficient. It appeared that the patient all through his life had had at least 1/4 litre of milk daily!! The parathyroid disorder had persisted for 36 years without the development of osseous changes! Similarly the case shows that an apparently normal serum phosphate level does not exclude the presence of hyperparathyroidism, because supervening renal disease may mask the abnormally low serum phosphate level. b) Determination of the TRP seems to be of little value, at least in the presence of complicating renal disease. c) The calcium tolerance test seems to be of great diagnostic value. d) On daily dose of 9 g sodium phytate and diet relatively low in calcium, the urinary excretion of that mineral could be kept as low as 50—150 mg daily This is remarkable in view of the fact that in patients with hyperparathyroidism on a diet as low in calcium as 150 mg daily the urinary excretion of calcium is usually estimated to amount to about 250 mg daily e) For more than 12 months the tendency to formation of renal calculi in this patient was strongly inhibited by continuous administration of 9 g sodium phytate daily The two last mentioned features (d and e) provide further evidence in favour of the concept that hypercalcaemia in hyperparathyroidism is not merely maintained by increased bone resorption, but is also to a considerable extent due to increased intestinal absorption of calcium. f) A convincing hypersensitivity to vitamin D could be demonstrated this is scarcely a generally recognized phenomenon in this condition It goes without saying that in this patient continuous long-term therapy with sodium phytate on a diet low in calcium would have involved a considerable risk of severe osseous changes at some future time.

Case 3 needs no comments. The prompt response to sodium phytate is strong evidence in favour of the suggestion advanced by Henneman et al. viz that vitamin D intoxication is an important indication for treatment with this drug

Summary

1 Oral sodium phytate counteracts the normal intestinal absorption of calcium by the formation of complex calcium salts. At the same time, the phosphate absorption is increased because of a partial hydrolysis of these salts. By these mechanisms it is possible to obtain a considerable depression of the calcium level in hypercalcaemic conditions.

2 The treatment given in three patients with hypercalcaemia is reported. One patient with generalised sarcoidosis and pronounced hypercalcaemia, prob-

by the impaired renal function, which remained essentially unchanged during the entire experimental period, but improved somewhat after the termination of treatment. As previously pointed out, the conspicuous effect of the treatment with sodium phytate obtained in this case was due to the blockage of one of the routes along which the blood content of calcium is renewed.

In case 2 the hypercalcaemia was difficult to classify Stone formation had occurred ever since the childhood of the patient. The serum levels of phosphate and alkaline phosphatase were normal The renal function was reduced to about 50 % possibly due to the hypercalcaemia itself but presumably also because of recurrent urinary tract infection Primarily the patient might well be presumed to suffer from hyperparathyroidism, although this on further consideration seemed less likely In spite of the long history of disease, there were no osseous changes, as should then have been expected. The TRP % was apparently normal during periods when no sodium phytate was administered. This observation also weighed against a diagnosis of hyperparathyroidism In this patient the hypercalcaemia was primarily classified as the so-called idiopathic infantile variety which is usually ascribed to an "endogenic vitamin-D-like effect or to hypersensitivity to vitamin D We were aware that this disease is rare, but at that time we did not know that cases of it with such a protracted course have presumably never been described In this case, the excretion of calcium was apparently normal, or only slightly elevated but yet large enough to give a tendency to stone formation.

On experimental administration of a single dose of 50 000 I U of vitamin D_2, the serum calcium increased promptly from 10.7 to 12 2 mg/100 ml. This reaction was suggestive of hypersensitivity to vitamin D and, to some extent, in favour of a diagnosis of idiopathic hypercalcaemia. The increase in serum calcium observed in this experiment must presumably be ascribed to resorption of calcium from the bones. The concurrent small increase in serum phosphate may be explained by a so-called masking of the second mode of action of vitamin D i. e. a suppressive effect on the parathyroid function Strangely enough, the urinary excretion of calcium did not increase in this experiment. A possible defect in the citrate formation in the plasma may explain this phenomenon.

It must be added that the lower but yet normal serum phosphate level observed in case 2 as compared with the slightly increased values in case 1 cannot be explained by a difference in the TRP % which was higher in case 2. The glomerular filtration was also higher in case 2 which may give a partial explanation of this phenomenon The discrepancy between the two patients correlated with the definitely decreased serum phosphate values which were gradually detected, and especially the less conspicuous effect of sodium phytate on the serum calcium led to a revision of the diagnosis. That the phosphate excretion in the long term experiment in case 2 was less than in case 1 may be explained by the presence of a smaller amount of phytase in the intestinal tract.

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On experimental administration of a single dose of 50 000 I U of vitamin D_2, the serum calcium increased promptly from 10 7 to 12.2 mg/100 ml This reaction was suggestive of hypersensitivity to vitamin D and to some extent, in favour of a diagnosis of idiopathic hypercalcaemia. The increase in serum calcium observed in this experiment must presumably be ascribed to resorption of calcium from the bones. The concurrent small increase in serum phosphate may be explained by a so-called masking of the second mode of action of vitamin D i. e. a suppressive effect on the parathyroid function Strangely enough, the urinary excretion of calcium did not increase in this experiment. A possible defect in the citrate formation in the plasma may explain this phenomenon

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Studies on Iron Absorption

By

Edvar Wolff Sorensen

Iron is absorbed as ferrous iron from the stomach, but also, and mainly from the upper part of duodenum (6, 11, 12, 25). Iron contained in the food is found in the ferric state. Hydrochloric acid frees some of this iron from the food, but is not necessary for its absorption (17, 25). The ferric iron must be reduced to the ferrous state before being absorbed, as only thus can pass though mucosal cells (11). The reduction occurs in the presence of ascorbic acid and other reducing substances (17, 19, 25). There is also evidence that ascorbic acid acts upon iron absorption and iron metabolism in more specific ways (16, 19).

It is known that components such as phytates, phosphates, eggs and bread interfere with iron absorption and there has been much discussion whether in hypochromic anaemias, oral iron should be given with or between meals to ensure highest absorption (9, 23). In his monograph Brise (2) has recently dealt with several questions regarding iron absorption. He has shown that the absorption was considerably higher when iron was given between meals, and that the absorption decreased by about 20—60 when it was given with meals. The same author as others, have shown that ascorbic acid increases the absorption of ferrous iron. This indicates that ascorbic acid has a direct influence on iron absorption which is independent of its reducing properties. The exact mechanism controlling iron absorption is still unknown — and the problems are far from solved (7).

The importance of carrying out iron absorption tests is discussed by several authors. Some of them such as Jasinski (13), Hauge (8), Heilmeyer (11), Heil and Keible (10) find it advisable and valuable to perform it. Others such as Laurell (15), Hahn et al. (6) are doubtful whether this examination is of value in the estimation of iron absorption. This is particularly so because the serum iron value at any time is a result of two opposite processes, the absorption from the intestinal tract and its transport from the blood to the iron stores. Many questions about these problems are still unsolved, but in the main they may be summarized as follows

Submitted for publication November 29, 1963.

ably induced by vitamin D was treated with sodium phytate for 16 months. The serum calcium level returned to normal, and the subjective symptoms fatigue and depression, disappeared A second patient with hyperparathyroidism in whom an erroneous diagnosis of idiopathic infantile hypercalcaemia was first made, was treated with sodium phytate for 14 months. Only a partial depression of the serum calcium level was obtained but a strong tendency to formation of renal calculi decreased considerably In a third patient with hypercalcaemia referable to an overdosage of vitamin D given to relieve parathyroid tetany prompt improvement was obtained by sodium phytate.

3 Sodium phytate (Rencal® Squibb Sodium Phytate) was given in three daily doses of 3 g in ice water The drug was well tolerated and no serious side effects occurred

4 It seems reasonable to assume that hypercalcaemia in sarcoidosis is usually induced by vitamin D therapy to which these patients (or possibly a special group of patients) seem to be very sensitive

5 In the patient with hyperparathyroidism, hypersensitivity to vitamin D was demonstrated Treatment with 9 g sodium phytate daily on a diet with a moderately limited calcium content resulted in an urinary calcium excretion of 50—150 mg daily No osseous changes occurred during the course of treatment. However continuous long term treatment with sodium phytate and a diet low in calcium in patients of this category presumably involves a considerable risk of the development of osseous changes, for which reason this therapy is absolutely contra indicated in such patients.

Acknowledgement

We are greatly indebted to Messrs. E. R. Squibb & Sons, New Brunswick, New Jersey U.S.A., who through their representative in Denmark, P M. Assens, cand. pharm., Manager of A/S Biofarma, Copenhagen, kindly supplied the drug for prolonged clinical trials in two of the patients.

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Table VI. Group 3. Results of iron absorption tests when the patients has been given tablets made of ferrous fumarate or ferrous succinate

Pat. no.	1st day				2nd day			
	Dose	Fasting value	Maxim. value	Maxim./Fasting value	Dose	Fasting value	Maxim. value	Maxim./Fasting value
1	F	22	126	104	N	14	98	84
2	N	52	191	139	F	57	78	21
3	N	59	108	50	F	35	128	93
4	N	23	309	280	F	24	135	111
5	N	17	224	207	F	18	52	34
6	F	34	72	38	N	37	179	142
7	F	107	265	158	N	63	200	133
8	F	55	98	43	N	58	112	53
9	F	62	123	61	N	58	184	126

F = 170 mg F⁺⁺ succinate N = 180 mg F⁺⁺ fumarate.

All values in µg %

Table VII. Group 4. Results of iron absorption tests on patients before and after gastric resection (Billroth II) has been performed

Pat. no.	Fasting state		2 hours		4 hours		6 hours	
	Before	After	Before	After	Before	After	Before	After
1	180	31	221	23	267	69	231	38
2	158	82	204	85	285	72	281	63
3	106	77	217	77	383	117	383	108
4	42	50	188	61	270	70	302	74
5	133	64	196	79	233	100	228	85
6	73	41	98	51	109	45	132	45
7	49	27	280	33	293	56	291	61
8	115	73	334	68	361	93	411	98
9	96	37	100	38	135	50	167	53
10	121	63	160	77	229	100	238	109
11	71	33	115	43	138	78	139	72
12	127	68	261	61	287	95	286	141
13	211	7	026	80	276	102	242	96
14	111	92	175	127	287	135	272	159

All values in µg

ferences between the fasting and the maximum values are entered. In addition a point scale is made. The day on which the highest serum level is observed, is given one point, the second highest 2 points, the third highest 3 points and the lowest 4 points. It is evident that the day on which the lowest sum of points is recorded reflects the day and type of test meal on which the highest iron absorption is obtained. The sum of the differences, as shown in table IV also demon-

Table IV A comparison of the rise of serum iron (i e. the difference between the maximum value and the fasting value) following the iron absorption test on each of four days trial. The results have also been transformed to a point scale. The day which reaches the highest value is given 1 point the second highest 2 points, the third highest 3 points and the lowest 4 points

Pat. no.	"Fasting day"		"Protein day"		"Fat day"		"Carbohydrate day	
	Value	Point	Value	Point	Value	Point	Value	Point
1	300	1	169	2	107	4	140	3
2	113	3	167	2	326	1	29	4
3	158	1	59	4	152	1	133	3
4	179	1	21	4	160	2	80	3
5	27	2	24	2	35	2	100	1
6	320	1	84	4	101	3	125	2
7	260	1	75	4	151	3	275	1
8	61	2	60	[illegible]	175	1	66	2
9	99	2	59	3	195	1	25	4
10	190	2	23	4	103	3	389	1
11	261	1	70	4	246	1	166	3
12	293	2	189	3	84	4	317	1
13	347	1	129	4	285	2	297	2
14	256	1	173	3	246	1	88	4
15	231	2	73	4	288	1	102	3
16	25	4	65	2	140	1	45	3
Total	3,120	27	1 440	51	2,757	31	2,377	40
Mean	195	1 7	90	3.2	170	1.9	149	2.5

Differences of ≤ 15 have the same point. All values in µg %

Table V The type of meal and the time during the iron absorption test, at which the maximum level of the serum iron is obtained. The numbers express number of patients

Meals	2 hrs	4 hrs	6 hrs
"Fasting day"	—	3	13
"Protein day"	6	4	6
"Carbohydrate day"	—	10	6
"Fat day"	—	3	13
Total	6	20	38

will not pass through the upper part of the duodenum. The iron absorption test was performed the day before operation and repeated on the day the patients left the hospital, i. e. approximately 12 days postoperatively

Results

Group 1 The reproducibility of the iron absorption test on the same subject is shown in table II The table shows two values for each patient on the same day first the fasting value and secondly the maximum. The variations from one day to another are very small.

Group 2 Table III shows the serum iron values, first in the fasting state and secondly the peak level after the oral iron dose given to each of the patients. The daily rise of serum iron level can be followed. It should be noted that the variations in fasting levels are small. The maximum levels in the same patient varies considerably from day to day This is more clearly shown in table IV where the dif

or the degree of saturation of the same. Because the break-down and absorption of protein, when compared to glucose, is rather slow this explanation is not very likely According to Cappel (3) it appears clearly proven that neither the total amount of transferrin nor the degree of saturation of the serum binding capacity influence directly the iron uptake from the gut.

2. The protein delays iron absorption and produces a flattened, peakless, curve shown in fig 1 In this way the total amount of iron may be the same in case and b, but this is not visible because the observations are only plotted for 6 hours. Study of table III and IV illustrates that this possibility is not likely From a physiological standpoint it is to be noted that, when given to a fasting subject, milk, fat and glucose will have passed beyond the stomach and upper part of duodenum within the first 3 hours.

3 That less iron is absorbed from the gut when it is given with protein than with the other meals. The tables shown strongly suggest that this theory is tenable. Radio-isotope studies may provide further evidence.

When iron is given to patients in a chelated form, as ironamminoate, the iron absorption tests show lower values than when ferrous fumarate has been used. In the former case no further protein break-down is possible, and the argument in favour of delayed absorption is even weaker These results are different from those reported by others. Rummel and Candon (22) have found amino-acids to be among the substances which favour iron absorption through the mucosal cells.

That insufficient iron absorption follows gastric resection is well known, and the reason is, following to Owren (21) "intestinal hurry" The great reduction in iron absorption illustrated in table VII is most probably the result of gastric content by-passing the upper part of duodenum. Because of the very short intervals between the two absorption tests it is improbable that other changes, such as mucosal atrophy have occurred. Chodos et al. (5) have found that the ability to absorb food iron is lost, or several impaired, after stomach resection, but that the ability to absorb iron salts is increased.

The results discussed above are in contradiction to a recent Lancet editorial which states that the effect of oral iron preparations entirely depends on the amount of iron administered and is not influenced by what the iron is attached to

Summary

The reproducibility of the iron absorption test is shown. The test has been applied on three groups of patients with sideropenic anaemia.

1 Oral administration of ferrous fumarate together with protein gives significantly lower serum iron values than when it is given with glucose or without any food at all

2. Oral administration of ferrous fumarate gives distinctively higher serum iron values than iron aminoate.

3 Oral administration of ferrous iron to patients with chronic peptic ulcer gives serum iron values reduced to approximately one third after stomach resection has been performed.

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Fig 1 A delayed iron absorption curve compared with one characteristic for sideropenic anaemia.
a = absorption curve "typical" for sideropenic anaemias.
b = a "delayed" curve.

strates the same findings. The table shows that the "protein-day" gives distinctly the highest sum of points and lowest sum of differences. None of the patients have their maximum level on the "protein day" but on the other hand 8 of the 16 patients have the lowest rise on that day. It is also interesting to see at what time after the test meal and test dose the absorption curves reach their maximum levels. Table V illustrates this. Of the 16 patients, 13 attain the maximum level after 6 hours on the "fasting-day" and the "fat-day" whilst on the "carbohydrate day" 10 patients reach maximum within 4 hours. On the "protein-day" the maximum values are almost equally divided between the three time intervals.

Group 3 Table VI shows that of 9 patients, 5 had a distinctly higher serum iron rise when given ferrous fumarate than when given chelated iron 3 patients showed almost similar response to the two drugs (difference ⇄ 25 µg%) and only 1 patient appeared to have the better response with chelated iron

Group 4 The results of the iron absorption tests before and after the gastric resection is shown in table VII There is a striking difference between the values for each patient. Generally speaking, the maximum level is reduced to a third of the maximum before surgery

Discussion

It is important to know whether or not an anaemic patient absorbs sufficient amounts of iron. The performance of an iron absorption test is a practical way to get information about this question. If the serum iron curve rises steeply after oral iron it indicates iron deficiency (1) This procedure has been criticised because the serum iron value at any time is a resultant of the amount of iron absorbed and of its transport from the blood to the stores. Factors which hasten or delay the absorption or the transport influence the final result.

It is shown in table II that the iron absorption curve on any patient is reproducible during stable conditions. By changing the type of food given together with the iron it is shown that the absorption varies considerably When iron is given together with protein rich food, the rise of the serum iron values is significantly less than when it is given with glucose or without any food at all. A fat meal gives an intermediate result. There is no relationship between these differences and gastric acidity The following explanations are possible

1 That the transport of iron from the gut to the stores is hastened when the iron is given with protein compared to when it is given with the other types of meal The idea behind this concept is that absorbed protein — and aminoacids may improve the transport system, i. e. the balance apoferritin ⇄ ferritin the release of ferritin from the gut wall the amount of transferrin present in the blood

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Arthritis Due to Salmonella Typhimurium

Report of 12 Cases of Migratory Arthritis in Association with Salmonella Typhimurium Infection

By

JUHANI VARTIAINEN and LEEVI HURRI

Arthritis is a known although rare complication of gastrointestinal infections such as ulcerative colitis, cholecystitis, infectious hepatitis, bacillary dysentery and brucellosis (3, 15). Likewise arthritis is known to occur in association with Salmonella infections. Arthritis has been reported to occur in 0.2 to 2.4 per cent of Salmonella infections (6, 7, 12, 13). S. cholerae-suis has been reported to cause the highest incidence, 2.4 per cent (12). David and Black (4) collected from the literature 84 cases of Salmonella arthritis, which in three cases had been caused by S. typhimurium. In Antila's (1) series of 139 cases of S. typhimurium infection treated at the Aurora Hospital during the years 1951–54 there was not a single case of arthritis.

Salmonella arthritis has been considered a suppurative bacterial process which is often associated with osteomyelitis. We have not found in the literature any definite reports on nonbacterial polyarthritis similar to our cases (a preliminary report has been published earlier (18)). When the present paper was in course of preparation, however, Berglöf's (2) report of seven cases of arthritis similar to ours was published. Of these, three had been bacteriologically confirmed by examination of faeces and all these three cases had occurred in association with S. typhimurium infection. Berglöf's cases resemble ours in every respect and the condition in question is obviously the same type of arthritis, to which little attention has hitherto been paid.

Material and methods

Our series comprises 12 patients in whom arthritis developed in association with S. typhimurium infection. In the present investigation all such cases treated at the Aurora Hospital and the Rheumatism Foundation Hospital during the period 1961–62 were considered. At the Aurora Hospital altogether 426 cases of Salmonella infection were treated

Submitted for publication December 3, 1963.

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Table II. Laboratory findings (maximal values)

Case no.	Phage type of S. typhimurium	Agglutinin titres for S. antigens ("Widal test")				AST	ASTA	Waaler-Rose	Latex	CRP	ESR (mm/h)	WCC (1,000)
		Somatic Group B	Flagellar									
			i	b	d							
1		500	150	—	—	64	2.2	—	—	2+	80	10.0
2	3a	—	50	—	—	64	0.9	—	—	3+	55	13.3
3	3a	500	500	—	—	80	5.0			—	20	11.2
4	3a	—	—	—	—	64	0.64	—	—	3+	131	20.2
5	3a	—	—	—	—	64	1.8	—	—	—	43	11.0
6	3a					32	1	—	—		91	16.2
7	1a var 1	50	—	—	—	280	6.4	64	—	—	68	7.5
8	1a	50	50	—	—	70	1.6	—	—	—	19	6.0
9	1a	50	—	—	—	110	2.2	—	—	—	37	13.7
10	3a	500	50	—	—	10	0.5	—	—	—	125	13.0
11	1	500	—	—	—	140	1.8	—	—	3+	120	21.0
12	3a	150	—	—	—	125	1.4	—	—	—	110	6.1
										Average	75	12.4

arthritis (both in 1943) which very much resembled the present disease. The symptoms had lasted for several months. During the intervening years the patients had been symptom-free.

Six months after the onset of arthritis, conjunctivitis and iritis occurred in case 4 and there was simultaneous exacerbation of the arthritic symptoms. The ophthalmologist in charge had considered the iritis rheumatic. After about one month the eye symptoms and the arthritis simultaneously subsided. At the time of follow-up the patient had been symptom-free for six months. This case also differs from the others in so far as the patient had not had diarrhoea or any other clinical symptoms of the Salmonella infection. When he was admitted to hospital for the arthritic symptoms S. typhimurium was found in his faeces. However, in other members of the patient's family S. typhimurium enteritis occurred simultaneously without symptoms of arthritis.

Laboratory findings (*table II*)

S. typhimurium was isolated from the faeces at least twice in all cases. In no specimens other than faeces could this bacterium be demonstrated. Bacillary dysentery (Shigellae) was not observed.

Phage typing was carried out (at the State Serum Institute, Rantasalo (10)) in 11 cases. Phage type 3a occurred in six cases, phage type 1a in three and phage type 1 var. 1 in one case.

The Widal test showed definitely elevated agglutinin titres for Salmonella antigens in four cases. The antistreptolysin 0 (AST) and Waaler Rose tests were negative; only in case 7 were there doubtfully positive values, AST 280 and Waaler Rose 64. The Latex test was negative in all cases. C-reactive protein (CRP) was found in the blood of four cases. The antistaphylolysin titre (ASTA) was elevated in two cases. The ESR at its highest averaged 77 mm/h and the white bloodcell count 12,400.

The synovial fluid was examined altogether six times in four patients. The fluid looked normal (serous) and cultures were negative.

X-ray examination of at least some of the affected joints had been carried out in all cases. Apart from swelling of soft parts and, in four cases, osteoporosis, nothing abnormal was observed.

Table I Clinical data

Case no.	Age (yrs)	Sex	Diarrhoea	Fever up to C	Duration of fever (days)	Duration of symptoms before arthritis (days)	Duration of arthritis (months)	Observation time (months)	Joints involved
1	48	♂	+	39.6	7	10	2.5	14	Left ankle, II finger
2	36	♂	+	38.4	10	6	3	12	Left knee and ankle, both wrists and hands, neck
3	15	♂	+	38.9	10	10	5	12	Right foot and ankle
4	54	♂	−	39.9	16	0	7	12	Right wrist, both knees, (eyes)
5	51	♂	+	38.2	?	15	3	12	Right foot and big toe, right shoulder left knee, sacro-iliac joints
6	34	♀	+	38.5	10	7	6	12	Left knee, both wrists
7	25	♀	+	38.5	2	10	6	6	Left ankle, fingers
8	25	♀	+	39.0	10	10	6	6	Left big toe, right thumb, sternum at the point of III rib left Achilles tendon
9	18	♀	+	37.8	36	9	5	6	Right ankle
10	10	♀	+	38.5	30	60	4	10	Right knee, III finger
11	22	♀	+	39.0	8	6	3.5	10	Right wrist, left knee and ankle
12	35	♂	+	38.0	?	8	5.5	11	Both knees, right ankle
			Average	38.7	12	12	5		

during the period 1961–62 and 329 of these were caused by S. typhimurium. Eight of our patients were treated at the Aurora Hospital and thus the incidence of arthritis is 1.9 % of all salmonelloses and 2.4 of th Salmonella typhimurium infections. No arthritis caused by Salmonellas other than S. typhimurium were observed.

In all our cases S. typhimurium was isolated from the faeces at least twice. Cases 1–8 were followed up in the out patient departments early in 1963 and patients 9–12 answered a written inquiry.

The most important clinical and laboratory data are given in tables I and II.

Clinical data (table I)

The ages of the patients varied between 10 and 54 years and there were equal numbers of male and female patients. The course of the disease was as follows. One to two weeks after the onset of diarrhoea with fever the patients had migratory arthritis, the duration of which was five months on average.

With the exception of one case (case 9) the arthritis was polyarticular. In most cases the knees and ankles were affected. A characteristic feature was the flitting nature with subsequent rapid relief and recurrences of the symptoms. When there was an exacerbation of symptoms there was often also recurrence of the fever. The main symptom was swelling and tenderness in the joints to lesser extent there was local reddening and heat. The swelling was usually periarticular. Two patients had an effusion of the knee joint. In these patients the fluid aspirated from the knee appeared normal and culture did not reveal any bacteria.

In conjunction with diarrhoea two patients (cases 4 and 6) had also previously had poly

seen previously described only in our preliminary report (18) of the present cases and in Berglöf's very recent paper (2). Berglöf's cases seem to be very similar to ours. All three of Berglöf's bacteriologically confirmed cases showed S. typhimurium infection and so did our 12 cases. This is no indication, however, that similar arthritis is absent in other types of salmonellosis rather the opposite would seem probable. The S. typhimurium infections constitute the bulk of our series: of the 426 cases of salmonellosis from the Aurora Hospital 329 were S. typhimurium infections. However, among the remaining 97 Salmonella infections of other types there ought to have been one to three cases of arthritis had the incidence been the same as in S. typhimurium infections.

Phage type 3a caused a comparatively widespread epidemic in and around Helsinki in the beginning of 1962 and seven of the present cases caused by this phage type occurred during this epidemic. However, phage type 3a does not seem to be more virulent than the others in causing arthritis, since the distribution of phage types in our material corresponds with their general distribution in Finland during 1961—1962 (9).

In the present series there was no case of suppurative Salmonella arthritis in which Salmonella could be isolated from the joint fluid.

It also seems as if there were some kind of individual disposition to arthritis in connection with intestinal infection, since two patients of the present series had earlier had similar arthritis in association with diarrhoea.

One of our patients had iritis and conjunctivitis in association with arthritis (although, in fact, not until six months after the onset of arthritis). It is interesting to note that Berglöf, too, reports one case in which, together with bacteriologically confirmed S. typhimurium infection, there was arthritis, urethritis and conjunctivitis. These cases seem to have much in common with Reiter's syndrome (3, 11).

It is obvious that in some cases of Salmonella arthritis an aetiological diagnosis cannot be made. Manifest diarrhoea may be entirely lacking or of such short duration as to escape attention. It would therefore seem advisable to carry out at least the Widal test and bacteriological culture of faeces in arthritis of unknown aetiology.

Summary

Twelve cases of polyarthritis occurring in conjunction with Salmonella infection bacteriologically confirmed by culture of the faeces are presented. In all these cases *Salmonella typhimurium* was isolated, while in connection with cases of other types of salmonellosis arthritis did not occur. The frequency was 1.9 per cent of all salmonelloses and 2.4 per cent of the S. typhimurium infections. The distribution of phage types of the isolated bacteria corresponds to their general distribution in Finland at the time of the investigation.

The clinical picture closely resembled that of rheumatic fever. Aspiration of the joints gave sterile, serous synovial fluid. X-ray examination did not reveal any destructive changes. AST and rheumatoid serological tests were negative. Arthritis set in on the average 12 days after the onset of gastro-intestinal symptoms and lasted five months on the average. Follow-up was carried out in all cases and since their recovery the patients have remained symptom-free from 6 to 14 months.

Therapy and prognosis

As a rule the patients had been treated with chloramphenicol, salicylates, phenylbutazone and/or corticosteroids. One patient had been treated with salicylates alone and the duration of his symptoms was 5 months, or the same as the average duration of symptoms. In the absence of a suitable control series, no conclusions with regard to the results of treatment can be drawn.

Complications

As already mentioned, one patient had iritis and conjunctivitis. Neither carditis nor nephritis was diagnosed in any of the patients.

Illustrative case

The clinical course of the disease is best illustrated by a case report. The case is typical and representative of the whole series.

Case 2. 36-year-old chaffeur who had previously been in good health. On Jan. 10, 1962, he fell ill with fever of 38.4° C and pain in different parts of the body. On Jan. 12 he had diarrhoea which continued for two days. On Jan. 15 the patient felt well and went to work. On Jan. 16, his temperature rose again and at the same time he felt pain in the neck and in addition pain with swelling in the left knee. On Jan. 17 the patient was admitted to the Aurora Hospital as a suspected case of meningitis. The spinal fluid was normal. Culture of the faeces produced S. typhimurium on several occasions. The rheumatoid serological tests as well as AST and ASTA were normal. At the time of admission to hospital the neck was tender and there was periarticular swelling and pain in the left knee. No effusion. On Jan. 20, the temperature returned to normal and the joint symptoms disappeared. On Jan. 26, there was again pain in several joints. There was also evident swelling of the right wrist and left ankle. The patient was subfebrile. A couple of days later the patient was again symptom-free. The ESR rose to 55 mm/h and the white blood-cell count at its highest was 13,500. In the hospital the patient was treated with chloramphenicol, salicylates, phenylbutazone and corticosteroids. He was discharged symptom-free on Feb. 10, 1962. At home the symptoms recurred and he had pain in the neck, in both wrists, in the fingers and the left knee in which swelling even occurred occasionally. The symptoms continued until April 1962. Since then the patient has been symptom-free.

Follow-up on Jan. 7, 1963. The patient was free from symptoms. The ESR was 6 mm/h. X-ray examination did not reveal anything pathological.

Discussion

The Salmonella arthritis cases reported in the literature have been either proved or been assumed to be cases of bacterial suppurative arthritis (3—6, 12—14, 16, 17, 19). Our cases seem to be of a different type. Instead of having suppurative arthritis they had (with one exception) polyarthritis. The swelling was, as a rule, periarticular, local reddening and heat being insignificant. Effusion occurred in two patients. Joint aspiration yielded a serous, sterile fluid. The clinical course of the disease greatly resembled that of rheumatic fever. Streptococcal infection had not preceded the condition but Salmonella infection had been established. AST was negative. There were no heart complications. The clinical picture was more acute than is generally the case in rheumatoid arthritis and the rheumatoid serological tests were negative. Similar cases of aseptic polyarthritis have been described in connection with other gastrointestinal infections such as ulcerative colitis, cholecystitis, infectious hepatitis, bacillary dysentery, brucellosis (3, 15) and in connection with genito-urinary infections (3, 8, 11). It is well known that in typhoid fever (3, 6) there are also sometimes joint complaints of short duration which are mostly subjective only. Aseptic polyarthritis in conjunction with Salmonella infections such as occurred in the present cases we have

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Myasthenia Gravis and Systemic Lupus Erythematosus

By

T. E. Mäkelä, R. Ruosteenoja, O. Wager, G. R. Wallgren and E. J. Jokinen

The literature so far contains reports of 10 cases in which myasthenia gravis was associated with systemic lupus erythematosus (S. L. E.) (1–15). Two cases have also been described in which S. L. E. developed following thymectomy for myasthenia gravis (1). A case of myocarditis, thymoma, and positive L. E. cells has been recorded (4).

In the following a case will be reported of co-existent myasthenia gravis and S. L. E. both with fairly typical manifestations. Thymectomy was performed and the thymus examined histologically.

Case report

A widow aged 51 occupied as packer has been under our observation since 1959. Since 1943, she has had variable joint symptoms in the hands and recently also in other joints, but with no impairment of working capacity.

In 1949–1950, the patient was subjected to prolonged hospital examination for leukopenia and anaemia. When lowest the leukocyte count was 1,600/mm^3 the differential count was neutrophils 12, eosinophils 7.5, monocytes 3, and lymphocytes 77.5 %; thrombocytes were 183,000/mm^3. One of the differential counts revealed 24 % eosinophils. At that time the blood changes were ascribed to chronic benzene poisoning, as she had been working as car painter for period of several years. Her subjective condition was good. The patient was advised to change her occupation.

At the beginning of 1955 the patient had several febrile periods with fatigue, headache and articular symptoms. In March and April 1955, she was hospitalized for five weeks and had slight elevation of temperature all the time. Mild granulocytopenia was established, the ESR was 27–48 mm/hour and there was distinct hypergammaglobulinaemia, 2.2 g/100 ml. Typical L. E. cells were noted several times in the peripheral blood. The case was considered to be a S. L. E., and ACTH and cortisone treatment was given. The patient regained satisfactory condition for period of four years, during which there was no medical supervision.

In early spring of 1959 after an acute respiratory infection, the patient first experienced muscular weakness in her extremities, masseter muscles and eyelids. She was admitted to Aurora Hospital for the first time. Response to neostigmine as well as the faradic current test before and after neostigmine injection indicated myasthenia gravis. A daily dose of 75–150 mg of neostigmine was prescribed and has since then enabled her to continue light manual work as a packer. No respiratory or swallowing difficulties have been experienced. Transverse tomography in 1959 and

Submitted for publication December 4, 1963.

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other parts of the thymus marked medullary and possibly even cortical hyperplasia was observed. The medulla contained a large number of germinal centres of varying sizes, the largest of which had distinct outlines. The germinal centres consisted of relatively small cytoplasmic cells with rather compact nuclei. Some of the cells showed degenerative changes, while the mitoses were few. The lymphocytes formed compact mass around the germinal centres. They were occasionally arranged in rows. Closer to the cortical area few plasma cells were seen. Hassall corpuscle were numerous, and were partly large and well outlined, partly more irregular. There were no mast cells in the thymus tissue: moderate numbers were seen in the connective tissue.

A lymph node removed during operation from behind the left clavicle showed normal follicular structure. The germinal centres were moderately large and well formed, and not very active. The mitoses were somewhat enlarged. There were large numbers of plasma cells in the lymph node and its capsule.

In biopsy specimen from the sternocleidomastoid the striated muscle was, on the whole, normal in appearance. A few sections showed small perivascular lymphocyte accumulations in the endomysial connective tissue.

Medication with prednisone was begun after operation. Three days after operation pulmonary oedema developed, with tachycardia of 180 and fall in blood pressure. Morphine was given, the patient was digitalized, intermittent positive pressure respiration was given, and anticoagulant medication was begun. Small doses of neostigmine were also given. The patient's condition improved during the following days. No respiratory distress, cardiac symptoms or marked muscular weakness were observed after the tenth postoperative day. The patient was discharged on October 25 in fairly good condition. A prednisone treatment of 10 mg daily was planned for the following month. On leaving hospital the patient had no need of neostigmine.

Discussion

The patient state conforms to the criteria of S. L. E. laid down by the Medical Research Council Collagen Disease Panel 1961 (11). Moreover typical L. E. cells were found in the patient's peripheral blood on several occasions, and anti-deoxyribonucleoprotein and anti-deoxyribonucleic acid antibodies were also detected. Thus the diagnosis of S. L. E. can be considered well established.

The simultaneous occurrence of myasthenia gravis and S. L. E. has been reported in a few cases only. Observations made in recent years suggesting the central role of the thymus in the control of immunity mechanisms and in the aetiology of the autoimmune diseases support the view that this association is *not* due to mere chance, but that myasthenia gravis and S. L. E. may have a common pathogenesis on an immunological basis.

A number of facts suggest that myasthenia gravis is an autoimmune state. In 60—70 per cent of myasthenic patients germinal centres, incomplete lymph follicles, and plasma and mast cell accumulations are seen in the thymus (13). According to Burnet's hypothesis, this histological picture is due to the emergence of cell clones reactive with self antigens and resistant to the normal homeostatic control mechanisms (2). The presence of multiple autoantibodies in myasthenia gravis also speaks in favour of the autoimmune character of this disease (5).

For a number of years S. L. E. has been considered an autoimmune disease. The histology of the thymus has been studied in very few cases of S. L. E. but some of these showed structures resembling germinal centres (9). One case has been described with concomitant S. L. E. and thymoma (7).

Certain common clinical features, such as the same precipitating factors, and similar sex and age distributions, also suggest a similar fundamental disturbance in myasthenia gravis and S. L. E. (12).



Fig. 1. Germinal centres in thymic medulla. × 40



Fig. 2. Germinal centre in thymic medulla. × 180.

1963 revealed a shadow behind the sternum possibly indicative of enlargement of the thymus.

In 1959 several indications of S. L. E. were also observed. The ESR varied between 60 and 90 mm/hour and hypergammaglobulinaemia and granulocytopenia were noted. L. E. cells were found. Symmetrical polyarthritis resembling that of rheumatoid arthritis was observed in the distal and proximal interphalangeal joints of the fingers. There were no signs of renal or cardiac involvement and no hepatomegaly or splenomegaly. Pulmonary roentgenograms revealed slight parenchymal fibrosis and thickening of the interlobar pleura.

In December 1962, the patient was admitted to hospital for fulminant haemorrhagic gastroenteritis, from which she recovered in one week. No salmonellosis or bacillary dysentery was noted. The findings of colon roentgenography and rectoscopy were normal. Routine roentgenographic examinations of the lungs revealed infiltration occuring in small spots all over the lungs. These changes had not been seen in 1959. Cultures for tubercle bacilli were negative. Various antibiotics elicited no response as regards the infiltrations, nor did prednisone therapy of three months duration. L. E. cells were again observed. Leukopenia was still present. Waaler Rose's test and the latex test were negative, antistreptolysin titre 160, antistaphylolysin titre 1:6, the direct Coombs test was negative. Positive seroreactions for syphilis (WR, WR sensitized with cholesterol) were obtained. TPI was negative.

In September 1963, the patient displayed articular symptoms more severe than ever before. She complained of distinct morning stiffness and tenderness on movement in all joints. Occasional swellings in the proximal and distal interphalangeal joints in the hands occurred symmetrically. In the roentgenograms these joints exhibited symmetrical changes such as are often seen in stage II of rheumatoid arthritis. The myasthenia had remained more or less unchanged.

At this time the following tests for antinuclear antibodies were performed:

1. The L. E. cell test (14) was repeatedly strongly positive.

2. Anti-deoxyribonucleoprotein antibodies were detected in undiluted serum by the fluorescent spot test (3).

3. Anti-deoxyribonucleic acid antibodies were determined by two techniques. In passive haemagglutination with tanned erythrocytes (10) and commercial DNA (Mann Assayed Biochemicals, New York, N. Y.) as antigen (6) the test was positive in titre 1:256 on several occasions. In passive haemagglutination with formalinized erythrocytes (8) and highly purified and polymerized DNA (Mann Research Laboratories, New York, N. Y.) as antigen the test was negative.

Thymectomy was performed on October 10, 1963. The extirpated H-shaped thymus weighed 25 g. In the body of the thymus, on the left, there was a cystic tumour 30 mm in diameter.

Histological examination (figs. 1 and 2) showed that the tumour was surrounded by a thin capsule of connective tissue. In the centre of the tumour there was a cyst containing mucous material and lined with columnar epithelium. In the rest of the tumour and in

Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Clinical Experiences with Ethacrynic Acid, a New Non-Thiazide Saluretic Agent (MK-595)

By

Gustav Schröder, Rune Sannerstedt and Lars Werkö

During the last few years many new potent saluretic drugs belonging to the thiazide group have appeared. The effects have been qualitatively the same, and the differences between them have mostly been in dose requirement, duration of action and price. Their side-effects have also been almost the same.

A new saluretic drug, ethacrynic acid (MK 595), developed in 1962 (8) represents a chemically new series of compounds with diuretic activity (fig. 1). In animal experiments it has shown a different mode of action from other diuretic agents. Bicarbonate excretion is suppressed in favour of a marked chloruresis (1, 2). The present report concerns a preliminary clinical trial with this agent.

Material

The drug has been given to 16 hospitalized patients. Some clinical data are given in table 1.

Group I consists of 7 patients who had been under recent treatment with diuretics, but where inadequate control of fluid retention or side-effects necessitated a *change of regimen*. Their ages ranged from 57 to 81 years. Six were in function group III–IV according to the criteria of the New York Heart Association. All patients were digitalized.

Group II comprises 9 patients aged 42 to 67 years, not recently treated with diuretic drugs. Eight belonged to function group III–IV. All patients were digitalized. One subject (O. W.) was uremic.

Performance

The patients were observed in the ward for several days before the treatment with MK 595 was started. Throughout the trial they got the ordinary hospital diet and had free fluid supply. The patients were weighed every morning before the first meal. The urine was collected in 24-hour portions and analyzed for its content of sodium, potassium and chlorides. Blood samples for determination of serum electrolytes and serum creatinine were taken before treatment with MK 595 and then as a rule twice a week.

In group I previously used diuretics were withdrawn when treatment with MK-595 was started, except in patient P. O., where chlorthalidone was continued. Digitalis and other drugs, including potassium chloride, were maintained. The patients in group II also continued the previous digitalis medication. In patient E. H. spironolactone was added later on.

Submitted for publication December 3, 1963.

Summary

A case is described in which myasthenia gravis and systemic lupus erythematosus were diagnosed in the same patient. The resected thymus showed marked medullary hyperplasia with a large number of germinal centres. Antinuclear antibodies were found in the serum.

Acknowledgement

Supported by research grant AM 03026-04 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

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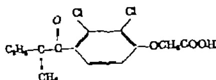


Fig. 1. Ethacrynic acid [2, 3-dichloro-4-(2-methylenebutyryl)phenoxy] acetic acid. $C_{13}H_{12}Cl_2O_4$

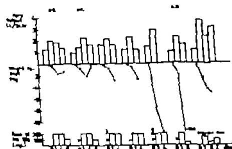


Fig. 2. Group I Effect of MK 595 on diuresis and weight reduction during treatment. B = the day prior to MK-595 therapy

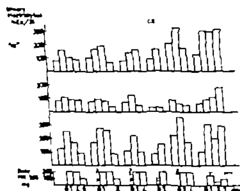


Fig. 3. Group I Effect of MK 595 on urinary electrolytes

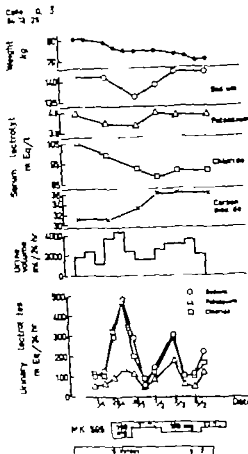


Fig. 4 Group I Effect of MK 595 on urinary electrolytes and body weight in case V O

the diurnal urinary volume decreased after 3—4 days. In three patients a further weight reduction of more than 5 kg was obtained after treatment for one week.

A marked rise in sodium and chloride excretion ppeared in the six patients analyzed, whereas there was no consistent change in the urinary potassium (fig. 3)

The most consistent change in the serum electrolytes was the increase (2—11 mEq/l) in carbon dioxide concentration found in all subjects. Th serum sodium concentration was unchanged. Four of the seven patients had a decrease in serum potassium concentration (0.2—2.1 mEq/l) The concentration of serum chlorides decreased in six patients, in four of them by more than 10 mEq/l (11—17 mEq/l)

Table I Clinical data. Group I recently treated with diuretics Group II no recent treatment with diuretics

Pat.	Sex	Age	Diagnosis	Function group acc. to N.Y.H.A.	Heart rhythm	Serum creatinine (mg%)	Recent treatment with diuretics in g/day
Group I							
A. S.	♀	59	Aortic and mitral valv. dis. + induced hypothyreosis	IV	SR	0.7	Chl. 1.0
V. A.	♀	62	Op. constrictive pericarditis	IV	AF	0.9	Chl.-thal. 0.1 + Hg-diuretics
V. O.	♀	57	Op. mitral dis. + diab. mell.	III	AF	0.9	Chl.-thal. 0.1
C. E.	♂	81	Art.-scler. heart dis.	II	SR	0.9	Chl. 0.25
L. A.	♂	73	Art.-scler. heart dis.	IV	SR	1.5	Chl.-thal. 0.1
A. B.	♀	69	Hyp. cardiovasc. dis.	III	SR	2.6	Chl. 0.5
P. O.	♂	68	Art.-scler. heart dis. + diab. mell. + nephrotic syndr.	IV	SR	1.2	Chl.-thal. 0.1
Group II							
F. J.	♀	54	Mitral dis. + art. hypertension	III	AF	0.9	–
A. L.	♀	42	Aortic and mitral dis. + induced hypothyreosis	IV	AF	1.3	–
K. K.	♀	62	Hyp. cardiovasc. dis. + diab. mell.	I	SR	1.1	–
B. D.	♂	67	Pulm. emphysema + cor pulmonale	IV	SR	1.0	–
E. H.	♀	56	Arteriovenous pulm. aneurysms	III	SR	1.2	–
K. P.	♀	58	Op. mitral dis.	III	AF	0.7	–
I. H.	♀	46	Op. mitral and aortic dis.	III	AF	1.3	–
O. W.	♀	64	Diab. mell. + nephrotic syndrome	IV	SR	7.5	–
A. H.	♀	56	Mitral and tricuspid val. dis. + pulm. embolism	III	AF	1.0	–

SR = sinus rhythm. Chl. = chlorothiazide. AF = atrial fibrillation. Chl. thal. = chlorthalidone.

The initial dose of MK 595 was usually 50 mg q. i. d. The dose was individually adjusted depending on the diuretic response and the weight reduction.

Methods

The urinary concentration of sodium and potassium was determined in an Eppendorf flame photometer. The urinary chloride concentration was measured according to Brun (3).

The serum concentrations of sodium, potassium, chloride and carbon dioxide were determined with the standard methods for a Technicon Auto Analyzer. The serum creatinine level was also measured in an autoanalyzer using a picric acid method according to Hollander (4).

Results

Group I. In all patients the diuresis increased and the body weight dropped during treatment with MK 595 (fig. 2). The diuretic response appeared during the first day of treatment in all. As a rule

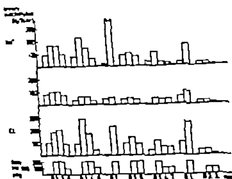


Fig. 6. Group II. Effect of MK 595 on urinary electrolytes.

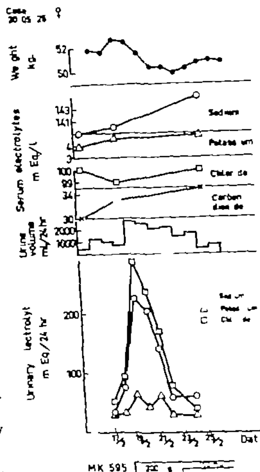


Fig. 7. Group II. Effect of MK 595 on urinary electrolytes and body weight in case A. L.

The urinary excretion pattern was characterized by a marked natri- and chloruresis, whereas potassium was excreted to a lesser degree thus creating a higher quotient between sodium and potassium excretion. The diuretic effect started on the first day of treatment in all patients.

Seven out of 16 patients had a reduction in their serum potassium concentration despite the small increase in kaliuresis. This may be harmful as apparently in the patient with the ventricular tachycardia.

Like other saluretic drugs MK 595 seems to produce a metabolic alkalosis. Thus in the present short-term study there was an increase in serum carbon-dioxide concentration in all patients. Similar serum and urinary electrolyte effects have been reported (4–6).

The effect on arterial blood pressure has not been especially studied in this trial. However, in one patient (K. K.) where MK 595 was given intermittently a marked blood-pressure drop from about 200/90 to 145/75 mm Hg was recorded after each dose. A hypotensive action less than that of thiazides is reported by Cannon et al. (4) in hypertensive subjects. Hemodynamic studies, however, have not revealed consistent changes in systemic arterial pressure, heart rate or cardiac output (7). Concerning the renal plasma flow and filtration fraction small inconclusive changes are reported (4–7).

Side-effects like nausea are reported (4) but no case of thrombocytopenia up to now.

The tentative value of MK 595 in the long term treatment of decompensated heart disease cannot be judged from this study. Our preliminary results show, however, that it has been possible to keep pa-

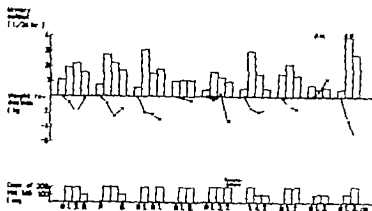


Fig. 5. Group II Effect of MK 595 on diuresis and weight reduction during treatment.

The effect of MK 595 is well demonstrated in fig 4 where also the dose responsiveness is shown.

Group II Six out of nine patients increased their diuresis and had a weight reduction (fig 5) One patient (E. H) had no further weight loss until spironolactone was added. Two patients (B. D O W) were unresponsive. As in group I the diuresis increased during the first day and decreased after 3—4 days of treatment.

Electrolyte excretions in the seven patients analyzed are seen in fig 6 The same pattern as in group I is seen i. e. a marked natri and chloruresis and only slight or no increase in potassium output.

The serum carbon-dioxide concentration increased by 2 to 8 mEq/l. The serum-sodium concentration was unchanged. The serum potassium and chloride levels decreased in three patients by 0.2 to 0 9 and by 8 to 10 mEq/l respectively

An illustrative case is presented in fig 7

No rise of serum creatinine was seen in either group during treatment.

Side-effects

One patient (A. H.) succumbed on the fourth day of treatment due to a large pulmonary embolus. The relation to the diuretic treatment is uncertain.

Severe hypopotassemia (2.5 mEq/l) might have provoked an attack of ventricular tachycardia in one patient (I H.) The previous heart rhythm was restored after intravenous infusion of potassium chloride.

Two patients suffered nausea that can be referred to the drug One patient got fever during treatment with MK 595 The clinical picture was that of a typical influenza common at that time. One patient (L H) got a rapidly reversed thrombocytopenia with 44 000 platelets/mm³ but without any bleedings.

Discussion

Even though this report deals with a small and heterogenous clinical material it is beyond doubt that MK-595 possesses a strong diuretic effect in patients with fluid retention of various etiologies. As judged from the results in group I MK 595 produced a good diuresis in badly decompensated patients where saluretics like chlorothiazide and chlorthalidone were unsatisfactory Similar findings are reported in the few papers published (4 6)

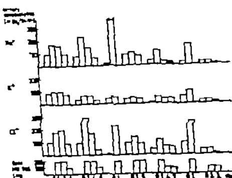


Fig. 6. Group II. Effect of MK-595 on urinary electrolytes.

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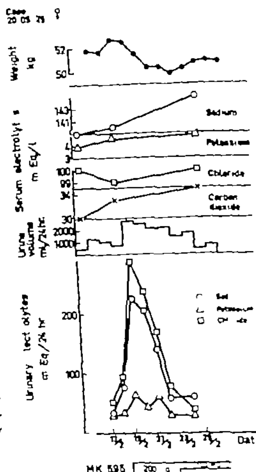


Fig. 7. Group II. Effect of MK-595 on urinary electrolytes and body weight in case A. I.

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Side-effects like nausea are reported (4) but no case of thrombocytopenia up to now.

The tentative value of MK 595 in the long-term treatment of decompensated heart disease cannot be judged from this study. Our preliminary results show however that it has been possible to keep pa-

tients previously needing complex diuretic therapy and frequent hospitalization periods on an ambulatory and simplified schedule with MK 595

Summary

1 The diuretic activity of MK 595 a new non thiazide saluretic drug has been studied in 16 patients with fluid retention of various etiologies. The drug was given orally in doses of 100—200 mg a day for 5—10 days

2 MK 595 produced increased diuresis in all but two patients. In seven patients, who previously were under continuous treatment with saluretic drugs MK 595 afforded a further marked increase in urinary output and reduction of edema.

3 The urinary excretion pattern was dominated by a natri and chloruresis while there was no conclusive increase in potassium in the urine. The serum potassium concentration was, however lowered in seven patients.

4 Hypokalemia may have been responsible for an attack of ventricular tachycardia. One subject developed a moderate thrombocytopenia. No other serious side-effects definitely attributable to MK 595 were seen.

5. MK 595 thus possesses a strong diuretic effect and might in many cases be effective where other diuretic drugs have failed.

Acknowledgement

Ethacrynic acid (MK 595) was supplied by Dr K. G Menzy Merck, Sharp & Dohme, New Jersey U.S.A.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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The Secretin Test in Patients with Haemochromatosis

By

GUNVOR PERMAN and ERNESTO BONERA

While the histological lesions of the pancreas in patients with haemochromatosis are well known the functional changes of the exocrine pancreas after stimulation with secretin seem to have been inadequately investigated. Dreiling and Janowitz (24) with their great experience in secretin tests (3 152 cases, 1962) have reported 4 cases of haemochromatosis (total 12 cases) with a bizarre response to secretin: enormous volume of secretion, low maximal concentration of bicarbonates and normal enzyme secretion. Churay and Bolgert (3) have found the same response pattern in one case of haemochromatosis and Marks et al. (15) have reported some cases of haemochromatosis with large secretion volumes after injection of secretin. No one however seems to have been able to give any explanation of the phenomenon and as very few cases have been described the matter appears to need further elucidation.

Submitted for publication December 10, 1962.

Material and method

The secretin test has been performed in 5 patients with haemochromatosis *with manifest* diabetes mellitus. The diagnosis has been established with clinical criteria, laboratory examinations and liver and skin biopsies. In all cases the diabetes was compensated and no patient had visible oedema or ascites.

Case 1. A 42-year-old man. *Liver cirrhosis* and diabetes mellitus. Liver biopsy and laboratory examination confirms the diagnosis of haemochromatosis.

Case 2. A 49-year-old man. Liver cirrhosis and diabetes mellitus. In addition to the *clinical and laboratory picture* skin and liver biopsies indicate haemochromatosis.

Case 3. A 41-year-old man. Liver cirrhosis and diabetes mellitus. Liver and skin biopsies *confirm the diagnosis of haemochromatosis.*

Case 4. A 40-year-old man. Typical haemochromatosis with confirmatory biopsies from the liver, skin and duodenal mucosa.

Case 5. A 52-year-old man. Typical haemochromatosis with confirmatory biopsies from the liver, skin and duodenal mucosa.

The secretin tests have been performed according to Lagerlöf (12). The duodenal con-

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The Department of Internal Medicine Karolinska sjukhuset, Stockholm, Sweden

Studies in Neurocirculatory Asthenia (Da Costa's Syndrome)

IV Course During Common Treatment and Physical Training and Relations Between Symptoms and Signs

By

Maj Levander Lindgren

The prognosis quo ad sanationem in neurocirculatory asthenia (NCA) is usually reported as not being good (4 8, 24 25, 26, 28, 29) Reassurance is commonly recommended as a therapeutic measure. Physical exercises are sometimes regarded as useless (28, p. 845) while physical training is reported to diminish the symptoms by Lewis (17) Hochrein (10) Lobanov (18) Olkhova (20) Chrastek (3) and Holmgren et al. (12, 13) In a preceding paper (15) the findings in the work test and orthostatic test in 130 patients with NCA were reported. Most of these patients were observed during prolonged periods of different treatment. In this paper a comparison is made between the course of NCA during routine treatment and during physical training A study is also made of the variations of findings in the work test and orthostatic test in relation to subjects symptoms.

Material and methods

One hundred and one patient were followed for at least one or two years after the first examination, and several patients participated in a follow-up examination or answered the questionnaire. The patients have reported whether symptoms, common in NCA (cf. 15) have vanished, decreased or persisted during periods of different treatment. As rule different subjects symptoms have changed in the same direction. Reports of variations with regard to different symptoms are given by Holmgren et al. (12, 13) In this paper the question of whether there is complete or incomplete recovery or no improvement is considered. Forty three patients with abnormal findings in the work test and orthostatic test were reinvestigated with these tests on at least one occasion.

Acute cases of NCA, caused by incidental factors, show a higher frequency of recovery than chronic cases (19 21 28) The patients in this series are therefore divided into two groups. 1) Acute cases in whom the history was less than two years (27 patients) 2) Chronic cases in whom the history was two years or longer (74 patients)

Routine treatment was heterogenous and comprised various drugs, attempts at psychological rehabilitation and varied advice with regard to physical activity given by different physicians. Several patients were sent for

Submitted for publication December 6, 1963.

A short report on this subject was given to the II Eur Congr of Cardiology 1960.

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Acta Medica Scandinavica. Vol. 175, fasc. 6, 1964

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Studies in Neurocirculatory Asthenia (Da Costa's Syndrome)

IV Course During Common Treatment and Physical Training and Relations Between Symptoms and Signs[1]

By

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The prognosis quo ad sanationem in neurocirculatory asthenia (NCA) is usually reported as not being good (4–8, 24, 25, 26, 28, 29). Reassurance is commonly recommended as a therapeutic measure. Physical exercises are sometimes regarded as useless (28, p. 845), while physical training is reported to diminish the symptoms by Lewis (17), Hochrein (10), Lobanov (18), Olkhova (20), Chrastek (3) and Holmgren et al. (12, 13). In a preceding paper (15) the findings in the work test and orthostatic test in 130 patients with NCA were reported. Most of these patients were observed during prolonged periods of different treatment. In this paper a comparison is made between the course of NCA during routine treatment and during physical training. A study is also made of the variations of findings in the work test and orthostatic test in relation to subjective symptoms.

Material and methods

One hundred and one patients were followed for at least one or two years after the first examination, and several patients participated in a follow-up examination or answered the questionnaire. The patients had reported whether symptoms, common in NCA (cf. 15) had vanished, decreased or persisted during periods of different treatment. As a rule different subjective symptoms have changed in the same direction. Reports of variations with regard to different symptoms are given by Holmgren et al. (12, 13). In this paper the question of whether there is complete or incomplete recovery or no improvement is considered. Forty-three patients with abnormal findings in the work test and orthostatic test were reinvestigated with these tests on at least one occasion.

Acute cases of NCA, caused by incidental factors, show a higher frequency of recovery than chronic cases (19, 21, 28). The patients in this series are therefore divided into two groups. 1) Acute cases in whom the history was less than two years (27 patients). 2) Chronic cases in whom the history was two years or longer (74 patients).

Routine treatment was heterogenous and comprised various drugs, attempts at psychological rehabilitation and varied advice with regard to physical activity given by different physicians. Several patients were sent for

Submitted for publication December 6, 1963.

[1] A short report on this subject was given to the II Eur. Congr. of Cardiology 1960.

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Table I Symptomatic improvement in neurocirculatory asthenia with "routine" treatment and physical training (Phys. tr) in acute and chronic cases

Treatment	Group	Total no. of pts.	Complete recovery		Relapse observed	Incomplete recovery		No improvement	
			No.	%	No.	No.	%	No.	%
Routine	Acute	20	6	30	1	8	40	6	30
	Chronic	71	4	5.6	2	27	38	40	56
	All cases	91	10	11	3	35	39	46	51
Phys. tr	Acute	9	6	67	1	2	22	1	11
	Chronic	32	2	6.3	1	28	87	2	6.3
	All cases	41	8	20	2	30	73	3	7.3

psychiatric consultation, some of them were given psychiatric treatment for periods with no more improvement on the average than with any other routine treatment. The group as a whole ought to be representative of the usual course in NCA, where periodic variations are common.

The physical training was supervised by the same therapist (F Mossfeldt) and followed a programme, detailed previously (12, 13) Some of the present patients are reported in these papers. A certain selection of cases resistant to routine treatment was made for the physical training, which is more time-consuming

Results with regard to subjective symptoms

A. Comparison between acute and chronic cases

A review of the material is given in table I The results were better in acute than in chronic cases with both treatments. In acute cases there was a tendency towards better results with physical training than with routine treatment, but the small number of cases does not permit definitive conclusions to be drawn.

In chronic cases 56 % of the patients did not improve after routine treatment but only 6.3 % failed to improve after physical training The difference between routine treatment and physical training is 49.4 ± 7.4 % ($P < 0.001$) The significance is given according to χ^2 analysis. Complete recovery had however the same low frequency with physical training as with routine treatment.

B Comparison between NCA somatic and NCA psychic

A division of the material is made in patients with dominating somatic symptoms and dominating psychic symptoms. (Acute cases and a small group with only single symptoms, NCA partialis (15) are not considered here.) The results were bad in NCA psychic as well as in NCA somatic after *routine* treatment (table II) With *physical training* the result was more favourable in both groups.

C Comparison between NCA with normal and NCA with low physical working capacity

During routine therapy there was no difference in prognosis between cases with

Table II. Comparison between the course with routine therapy and physical training (Phys tr.). The cases are divided with regard to clinical picture (NCA with dominating somatic and dominating psychic symptoms) and physical working capacity (normal and low PWC). Only chronic cases are considered

Treatment	Group	Total no. of pts.	Complete recovery		Incomplete recovery		No improvement	
			No.	%	No.	%	No.	%
Routine	Somatic	41	3	7	18	44	20	49
	Psychic	30	1	3	9	30	20	67
Phys. tr.	Somatic	20	1	5	17	85	2	10
	Psychic	12	1	8	11	92	0	0
Routine	Normal PWC	38	3	8	13	34	22	58
	Low PWC	33	1	3	14	42	18	55
Phys. tr.	Normal PWC	13	1	8	12	92	0	0
	Low PWC	19	1	5	16	84	2	11

capacity (PWC) (for definition see (15)) (table II). With physical training the results were markedly better in both groups. The difference between routine therapy and physical training with regard to patients with no improvement is statistically significant. In patients with normal PWC 58 % ± 11.4 (P < 0.001) and in patients with low PWC 44 % ± 11.6 (P < 0.01).

The material was also divided into groups with regard to the number of findings in the work test and orthostatic test (depression of the S—T segment and flattening of the T wave at rest, after work or in the standing position, high pulse rate in the standing position and low PWC). No difference with regard to prognosis appeared between these groups. Six cases with normal findings in the work test were trained satisfactorily, one with complete and 5 with incomplete recovery.

Drugs

Treatment with drugs can hardly be avoided in NCA, and a brief review of the drugs given in this series seems necessary. Sometimes the same drugs as before physical training were continued during the training, but, of course, no new preparations were started during the training. Sedatives and tranquilizers are commonly used in these series as in others (1, 7, 8, 9, 27, 28, 29). They break the vicious circle formed by anxiety and somatic symptoms.

Next to sedatives drugs with vegetative effects are most used. Ergotamine derivates, often in combination with vagolytic agents and sedatives, are reported to have a fairly good effect (1, 3, 6, 19, 22, 23). Such preparations have also been used in these series, but the expectations are often not maintained and good results at the start often vanish after some period of treatment. The mechanism is difficult to make out as ergotamine has complex cardiovascular effects with vasoconstriction as well as adrenergic blocking. A relief of troublesome peripheral coldness is often achieved by benzylimidazolin.

Nikethamide is another drug, which in the author's experience is of great value

Table III Variations in connection with recovery of "sympathicotonic" depressions of the S-T segments and flattening of the T waves at rest and after work in 43 NCA cases. The material is divided in groups with regard to therapy (physical training and routine therapy) and degree of recovery (complete or incomplete)

Group	Total no. of patients	ECG at rest					ECG reaction after work				
		Worst condition	Optimum condition				Worst condition	Optimum condition			
		Changes of the S-T segment and the T wave present	More pronounced or new changes	Unchanged	Less changes	Normalized	Abnormal react. of the S-T segment and the T wave	React. more abnormal or new changes	React. unchanged	React. less abnormal	React. normalized
		No. of patients									
Phys. training	27	8	0	1	0	7	13	0	3	4	6
Routine therapy	16	7	0	1	1	5	8	2	2	1	5
Complete recovery	9	2	0	0	0	2	3	0	0	0	3
Incomplete recovery	34	*13*	*0*	*2*	*1*	*10*	*18*	*2*	*5*	*5*	*8*
All material	43	15	0	2	1	12	21	2	5	5	11

in NCA. Nikethamide is known as a respiratory stimulant, and the symptomatic relief during attacks of breathing difficulties may be explained by this effect. A pronounced relief of precordial pain resistant to other drugs such as sedatives and ergotamine, was often observed also especially after intravenous and intramuscular injections. As the pathogenesis of this pain is unknown, discussions on this effect will be still more hypothetical.

Treatment of arrhythmias even benign premature beats, must not be neglected in NCA as they are often of etiological significance in perpetuating the disease.

Correlation between variations of symptoms and signs

Forty three patients with abnormal findings in the work test and orthostatic test were investigated during periods with symptoms of varying degree. In tables III IV and V the findings in the period with most pronounced subjective symptoms ("worst condition") are compared with the findings during complete or incomplete recovery ("optimum condition) At recovery there was as a rule a partial or total regression of the signs.

ECG changes (depression of the S—T segment and/or inversion or flattening of the T wave of "sympathicotonic type) *at rest* never worsened (table III)

Table IV Variations connection with recovery of depressions of the S-T segment and flattening of the T wave and high pulse rate during orthostatic test in 43 patients with NCA (see further table III and text)

Group	Total no. of patients	Depression of the S-T segment and flattening of the T wave: Worst condition	Optimum condition				High pulse rate: Worst condition	Optimum condition			
		Marked changes present	More marked changes	Unchanged	Less marked changes	Normalized	Present	Higher rate	Unchanged	Lower rat	Normal rate
		No. of patients									
Phys. training	27	19	1	2	5	11	20	2	2	3	14
Routine therapy	16	10	1	1	6	3	10	3	1	5	4
Complete recovery	9	4	0	0	2	2	6	0	0	2	4
Incomplete recovery	34	25	2	3	9	12	24	5	3	6	14
All material	43	29	2	3	11	14	30	5	3	8	18

ECG changes after work were more marked in 2 patients only in pite of the higher work load which was, as a rule, performed at the optimum condition.

One of these cases was the only patient, who deteriorated during training. She started ith relatively slight symptoms precordial pain, throbbing aching of arms and legs, and PWC was low During the physical training, hich was probably too intense, PWC became normal and ECG changes anished but her symptoms increased. The training was stopped, and the symptoms as well as PWC returned to their original status. This patient is therefore grouped as in optimum condition during routine treatment, though the findings in the work test and the orthostatic test were more marked

Marked ECG reaction and pulse reaction in *the standing position* became less marked or normal in most cases (table IV) but was observed to increase in a few cases. The *PWC* as a rule increased with improvement (table V) and was then in the majority of cases within normal limits. The a erage increase was 52 per cent from 460 kpm/min. to 700 kpm/min. As is to be expected, the PWC increased more when t was low from the star (from 410 to 680 kpm/min., i. e. 66 than when it was normal (from 560 t 740 kpm/min., i. e 32)

Comparison between complete and incomplete recovery

Only nine patients made a comple recovery (tabl III—V) ECG t r and after work and PWC were th

A highly schematic drawing has been made (fig 1) based on these therapeutic observations and earlier referred clinical and experimental experiences regarding *the etiology and pathogenesis of NCA* (16) Its aim is to serve as a work hypothesis and to illustrate the possible effect of physical training and the vicious circles formed in the condition NCA. The absence here of studied signs at work test and orthostatic test in particular in NCA with dominating psychic symptoms indicates that the disease may sometimes arise essentially at the psychic level. Most cases, however show signs indicating vasomotor vegetative disturbances. They are probably released by abnormal psychogenic stimulation of the vasomotor centre. This type of vasomotor reaction corresponding to "fright and flight reaction is called here "Stress adaptation in contrast to the vasomotor reaction during physical work called here Work adaptation Common vasomotor vegetative signs during stress adaptation are tachycardia blood pressure elevation sympathicotonic orthostatism, ECG changes of sympathicotonic type and sometimes a hyperkinetic circulation (vasoregulatory asthenia) This is characterized by high minute volume low arterio-venous oxygen difference and a low PWC (12) These signs are summarized as "Vasomotor vegetative NCA signs in fig 1 It is evident that these circulatory disturbances contribute to the patients subjective symptoms and form a *vicious circle.* During adaptation to work there is also an increase of minute volume but at the same time a marked increase also of arterio-venous oxygen difference. Physical training stimulates work adaptation which counteracts the stress adaptation as exemplified by arterio-venous oxygen difference. A favourable effect of physical training on vasomotor disturbances in hypertension is also observed by Klutnik (14)

Physical training has *psychological* effects also The regulatory supervision of an understanding therapist, the fellowship in training groups with other people with the same symptoms and problems, are in themselves therapeutic aids. The patients notice with increasing astonishment what feats they perform without harm to their hearts. This convinces them more than anything else, that they really have no heart disease. This explains the favourable effect on the symptoms even in patients without vasomotor vegetative disturbances during work test and orthostatic test. It lessens the tendency of emotional tension to evoke NCA symptoms seemingly more effectively than routine treatment including sedatives. Physical training thus means psychological as well as physical rehabilitation.

Summary

The course of neurocirculatory asthenia (NCA) was followed in 101 cases. In 3/4 of the patients it was chronic, sometimes with regressions and relapses. After "routine treatment 56 per cent of the chronic cases failed to improve after physical training the result was significantly better only 6 per cent failing to improve. The number showing complete recovery was however not influenced being about 6 per cent.

The favourable effect of physical training on the improvement of symptoms was observed in patients with dominating somatic as well as dominating psychic symptoms and with low as well as normal physical working capacity

In 43 patients the relations between symptoms and signs in the work test and orthostatic test in connection with im-

provement were studied. As a rule there was a simultaneous regression of both symptoms and signs, independent of the kind of treatment. Physical training was more effective than routine therapy in increasing the physical working capacity and counteracting the sympathicotonic orthostatism.

The discussion deals with the physiological and psychological effects of physical training and the varying combinations of symptoms and signs observed. Based on the experiences especially with regard to physical training in the present series of papers some features in the pathogenesis of symptoms and signs in NCA are hypothetically outlined and illustrated by a highly schematic drawing

To sum up, physical training cannot cure NCA but seems to produce a fairly good physical and psychological rehabilitation. It is highly effective in counteracting abnormal findings in the work test and orthostatic test, which are reflecting vasomotor vegetative disturbances caused by the condition of NCA.

Acknowledgements

To professor T. Sjöstrand, head of the Laboratory of Clinical Physiology, Karolinska sjukhuset, and his staff, I want to express my sincere thanks. All orthostatic tests and work tests and heart catheterizations were performed in his laboratory. He also aided this series of papers by stimulating discussions. I am also much obliged to Folke Mossfeldt who conducted the physical training.

Such was supported by grants to the Laboratory of Clinical Physiology from "Folksams rehabiliteringsfond" and "Svenska nationalföreningen mot tuberkulos", Stockholm. This investigation was further supported by a scholarship from "Axel Hirsch Donationsfond".

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